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Cardiac Tissue Regeneration; The Use of Stem Cells

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1. Introduction

Cardiovascular disease is the leading cause of illness and fatality. Diseases such as coronary artery disease, myocardial infarction and subsequent congestive heart failure pose a serious problem to much of the population in the Unites States and all over the world. The heart's compromised function that results from the above conditions is so influential as the heart has little capacity to repair its lost tissue; once the cardiac myocytes are destroyed its effects are amplified by a drastic decrease in cardiac function.

Until recently with the advent of stem cell technology, researchers believed that once cardiac myocytes are destroyed they could never be regenerated and thus little could be done to reverse the adverse effects of cardiac muscle atrophy (Laflamme and Murry 2005). This dogma, that the heart is a post mitotic non-regenerating organ and that cardiac myocytes are terminally differentiated cells that participate in cardiac function all throughout life, introduced more than 60 years ago, is being challenged as the results of different experimental and clinical work are showing capacity for cardiac tissue hyperplasia This paper will explore the various kinds of stem cells used in an attempt to regenerate cardiac tissue.

2. Stem Cells

Stem Cells are self renewing cells that characteristically can differentiate into other types of cells. Hematopoietic stem cells, adipose-derived stem cells, muscle-derived stem cells, cardiac stem cells and embryonic stem cells are some kinds of stem cells currently being studied in their use as cellular therapy for myocardial regeneration.

A. Hematopoietic Stem Cells

Hematopoietic stem cells (HSCs) are bone marrow derived stem cells. These cells have been the target of tissue engineering and cellular therapy as they are self renewing cells that can differentiate into multiple cell lineages (Wu et al 2006). HSCs exhibit great potential in developing into cardiomyocyte-like cells as blood and cardiac tissues originate in adjacent areas in embryonic development. In early development, blood cells originate in the mesoderm: the hematopoietic precursor cells accumulate in the blood until it finally reaches the bone marrow. The heart muscle is generated within the anterior lateral mesoderm. Originating from adjacent

regions of the mesoderm in the developing embryo is what contributes to these stem cells potency (Leri et al 2005).

Clinical work with the importation of bone marrow cells to the infarcted heart to regenerate cardiac cells has yielded varying results. Orlic and colleagues (2001a) transplanted bone marrow cells into the infarcted hearts of mice to see if the dead myocardium would be restored. The researchers sorted Lineage -, ckit+ bone marrow cells from mice and implanted them into the contracting wall bordering the infarct shortly after coronary ligation. Indeed the Lineage-, ckit+ cells differentiated into myocytes and formed vascular structures occupying 68% of the infarcted portion of the ventricle nine days after transplantation.

Similar to Orlic, Balsam et al (2004) also studied the effects of implanting Lineage - c kit+ hematopoietic stem cells expressing green fluorescent protein (GFP+), isolated from mice into the ischameic myocardium of wild type mice. Abundant GFP+ cells were detected in the myocardium ten days after the implantation, but by 30 days post implantation few GFP+ cells were detected. Balsam's work suggests that the HSCs remain in the infarcted region of the heart for a limited amount of time. Unlike Orlic's findings, Balsam discovered that the GFP+ cells did not express cardiac tissue specific markers, but rather, most of them expressed the hematopoietic specific cell marker CD45, indicating that these cells never differentiated into cardiomyoctes. Murry et al (2004), in his work tracked the fate of hematopoietic stem cells with 145 HSC transplants into normal and injured adult mouse hearts and found that transdifferentiation into cardiomyocytes was undetectable.

Researchers have also been investigating the mechanism for the therapeutic effects of HSC transplantation – primarily via the paracrine and cytokine signaling. Korf-Klingebiel et al's (2008) work with bone marrow stem cells sheds lights on the paracrine signaling between transplanted bone marrow stem cells and resident cardiomyocytes in the hosts's cardiac tissue. In their experiment, this research group shows that HSCs secrete proangiogenic, cytoprotective growth factors and cytokines thus promoting angiogenesis and cardiomyocyte survival via paracrine effects. Orlic et al on a search to find a noninvasive method implementing HSCs, to recover the injured heart found that transdifferentiation of primitive bone marrow stem cells is dependent on tissue damage and the level of pluripotent cells. He suggests that HSCs, mobilized by stem cell factor and granulocyte-colony stimulating factor, would home to the infarcted region, replicate, differentiate and promote myocardial repair. In his experiment, cytokine mediated translocation of bone marrow stem cells resulted in a significant degree of tissue regeneration. Cytokine induced cardiac repair decreased mortality by 68%, infarct size by 40% and diastolic stress by 70%. Cardiac ejection fraction also increased as the consequences of the formation of new myocytes with arterioles and capillaries connected with the circulation of the unaffected ventricles (2001- b).

Jackson et al (2001) tested a side population of CD34- bone marrow stem cells for their capacity to regenerate cardiac myofibrils in ischemically injured cardiac tissue. In his study, the side population cells and their progeny became incorporated into both cardiac muscle and vessel structures where they displayed the characteristics of differentiated cardiomyocytes, suggesting that the stem cells do differentiate into cardiomyocytes. While Jackson's study seems interesting, it cannot be taken as evidence that hematopoietic stem cells take on the cardiac cell phenotype after engraftment as his method used for isolating the side population CD34- bone marrow cells

appears to be lacking and thus there is much possibility that contaminating non hematopoietic stem cells in his experiment is what contributed to the cardiac cell engraftment.

The findings of Orlic and his counterparts have motivated researchers to investigate the effects of HSC injections in the post infarcted human heart. Several clinical trials have explored the hypothesis that an intracoronary infusion of these bona marrow cells may enhance recovery of Left Ventricular (LV) systolic function following Acute Myocardial Infarction (AMI). Meyer et al (2006) studied the specific effects of bone marrow cells on the recovery of left ventricular (LV) function in patients after AMI. His group learned that hematopoietic bone marrow cells did not significantly increase the long term systolic ventricular function of the post infarcted heart. He administered a single dose of HSCs to 30 of 60 patients undergoing post infarction therapy. In the control group LV ejection fraction increased by .7- 3.1 percentage points after 6 and 18 month respectively; LV ejection fraction in the bone marrow cell transfer group increased by 6.7 and 5.9 percentage points. Meyer noted that the difference in LV ejection fraction improvement was significant after six months but not after 18 months. While the single dose of HSCs did not provide long term benefit to left ventricular systolic function, the accelerated ejection fraction recovery rate after AMI due to HSC therapy was beneficial to the patient recovery.

Schaninger et al (2006) has obtained similar results to Meyer's in his clinical work. His group found that the recovery of left ventricular systolic function in patients after AMI is enhanced by an infusion of bone marrow stem cells into the reperfused coronary artery. Clinical data obtained by Janssen et al (2006), in their controlled experiment of 67 patients, indicate that HSC transfer when applied early after coronary reperfusion may lead to a reduction in infarct size and a better recovery of regional systolic function. Other studies, however, indicate that improvement in left ventricular function after intracoronary injection of bone marrow cells in the AMI is not seen (Lunde et al 2006).

Although the ability for hematopoietic stem cells to differentiate into cardiomyocytes is debated by different researchers, the use of these stem cells for myocardial regeneration nevertheless seems promising. Varying results have been obtained when injecting HSCs into the hearts of humans, yet most studies indicated a short term improvement in systolic function post cardiac trauma. The mechanism for these stem cells, primarily through a cytokine/paracrine signaling pathway can have great implications in the world of non invasive stem cell therapy.

B. Adipose- derived stem cells

These stem cells, derived from mammalian adipose tissue, recently displayed their ability to differentiate in to multiple lineages. In 2001, investigators from UCLA and University of Pittsburg demonstrated that a population of cells derived from collagenase digested human adipose tissue could be induced into multiple cell lineages including cardiomyocyte lineage (Zuk et al 2001). The ease of access to fat and its abundance makes adipose tissue a potentially useful source of stem cells for cardiac tissue regeneration.

Interestingly, adipose -derived stem cells exhibit close associations with bone marrow -derived stem cells. The cell surface phenotype of adipose -derived stem cells is almost identical to that of bone marrow -derived stem cells and thus their patterns of differentiation should seemingly be similar, yet the data of Winter et al (2003) suggests that in vitro differentiation of adipose- derived stem cells in mice is less than observed in bone marrow derived stem cells. According to these findings, the rate of differentiation of adipose- derived stem cells is much

slower than in hematopoietic stem cells. In absolute incongruity to these finding, a comparative study of marrow and adipose derived cells obtained from the same donors, performed by De Ugarte et al (2003) demonstrates that the adipose derived cells require approximately 5% the cell number used for marrow cells in order to reach initial confluence by one week. De Ugarte's work suggests that the proliferative capacity for adipose derived stem cells far exceeds that of hematopoietic stem cells. Their seemingly high proliferative capacity and their similarities to bone marrow derived stem cells signify the potential for adipose tissue to act as an alternate, perhaps preferred cell source for clinical application.

To date there hasn't been much clinical work in the area of using adipose derived stem cells to regenerate myocardium in vivo, yet some studies on the cardiomyocyte differentiation capacity for adipose derived stem cells have been published. Planat-Benard et al (2004) investigated the emergence of cardiomycyte phenotype from adipose derived cells and found that beating cells with cardiomyocyte features were identified after culture of the adipose cells. The cardiomycoyte phenotype was identified with expression of specific cardiac markers, immunocytochemistry staining, and ultrastructural anaylsis. The early culture also displayed a pacemaker activity in the cells. This study is important as it shows that functional cardiomyocyte-like cells could be directly obtained from adipose tissue. The potential for adipose tissue to function as a reservoir of stem cells that can differentiate into cardiomyocyte-like cells must further be investigated.

C. Skeletal muscle- derived stem cells

Skeletal muscle derived stem cells, also known as myoblast or satellite cells are being investigated in their potential to enhance cardiac function post acute myocardial infarction(AMI). These cells that lie under the basal membrane of skeletal muscle fibers, seem promising in the area of cell based therapy due to their preclinical efficacy, availability, ability to be amplified in vitro and their relatively good survival after implantation (Menasche et al 2003). Unlike other stem cells, satellite cells retain their specific characteristics and do not transdifferentiate into cardiomyocyte cells when transplanted into cardiac tissue (Reineke et al 1999). Still the myoblast's inherent contractile ability can be a great asset in improving the contractile capacity of scar tissue formed after cardiac injury. Several groups of researchers have investigated the effects of muscle derived stem cells injected into a post injury cardiac scar.

Murry et al (1996) tested the functionality of skeletal myoblasts in injured cardiac tissue via the injection of neonatal skeletal muscle cells into the the hearts of rats directly after coronary injury. At day three after implantation, myotubes expressing fast fiber, easily fatiguable MHCs were present in the injected cells. By week seven, the grafts began to express beta MHCs – a hallmark of the fatigue resistant, slow fiber phenotype. This continued until three months, yet all the while the grafts never expressed cardiomyocyte specific alpha MHCs. Murry reports that even though the grafts did not express a cardiomyocyte phenotype, they were able to contract; upon stimulation ex vivo; wounds containing two week old myoblast grafts contracted. Further, the myoblast grafts could perform a cardiac duty like cycle for six minutes, alternating between relaxation and contraction. Murry's experiment on how myoblast grafts convert to fatigue resistant slow twitch mature myofibrils are one of many such studies and supports the idea that fully developed myofibrils within the cardiac infarct retain their skeletal lineage and do not differentiate into cardiac cells with intercalated discs and other cardiac features.

Taylor et al (1998), in their study found that skeletal myoblasts can differentiate into striated muscle cells within damaged myocardium and these cells augment both systolic and diastolic myocardial performance after transplantation in the damaged myocardium. Taylor's group transplanted skeletal myoblasts into the infarcted myocardium of rabbits, monitored cardiac function in vivo for two to six weeks and examined serial sections of the hearts by light and electron microscopy. Islands of different sizes comprising elongated, striated cells that retained characteristics of both skeletal and cardiac cells were found in the cardiac infarct. Taylor's findings support Murry's claim that the skeletal muscle stem cells retain their skeletal muscle lineage. His study further indicates that in rabbits in which myoblasts were incorporated, myocardial performance was improved.

Similar to Taylor et al, Ghostine et al (2002) studied the effect of skeletal myoblast transplantation on a scar in the infarcted heart of a sheep. Ghostine's group injected myoblasts into the scar and studied subsequent cardiac function at 4 months and 12 months after the injection. His findings show that for up to one year the skeletal myoblast implantation limits post infarction ejection fraction deterioration, and improves systolic scar function by colonization of skeletal muscle cells in the fibrotic tissue. Although Ghostine's results are promising, they can't be validated as this clinical trial involves only one sheep and thus the indicated improvement in cardiac function can stem from various different sources. The true relationship between the skeletal myoblast transfer and greater ejection fraction must be further investigated.

The question of whether skeletal muscles beat in vivo in humans is a newly researched area that is currently being explored. Menasche et al (2008) began a phase I clinical study on the implantation of autologous skeletal myoblasts in post infarction scars in humans. Menasche and his group, veterans in the field of skeletal myoblast implantation, have previously found a linear relationship between the improvement in ejection fraction and the number of injected skeletal myoblasts after transplantation. In 2001, they reported the first human case study of implanting autologous skeletal myoblasts into a post infarction scar during coronary artery bypass grafting. Five months after the implantation, evidence of contraction and viability in the grafted scar by echocardiography and positron emission topography were obtained. (Menasche et al 2001) Later, in 2003, Menasche tested the safety and efficacy of skeletal myoblast implantation in patients with severe ischemic heart disease. Of the ten patients included in his trial, four developed ventricular tachycardia and were implanted with an automatic internal cardioverter- defibrillator, a device to regulate their heart beat (Menasche et al 2003). This could have been predicted with Murry's finding that the satellite cells have an inherent contractile ability While Menasche concludes that his clinical trial demonstrates the feasibility and safety of skeletal myoblast transplantation as his patients had an uncomplicated post operative course, this cannot be viewed uncritically. Although three of the four patients that developed cardiac arrhythmias had preoperative ventricular hyperexcitability the numbers seem very high; almost half the patients involved in this clinical trial developed this severe side effect.

Although in Ghostine et al's experiment (2002), myoblast transplantation has been found to limit post infarction ventricular dilation this was not seen in the Menasche's patients. The probable reason for this is that Menasche's patients that had previous old infarctions had already enlarged ventricular dimensions. In his clinical follow up in 2008, Menasche gathered 98 patients, with either left ventricular dysfunction, or myocardial infarction and an indication for coronary surgery. Each patient received an injection of either 400, or 800 million cells grown from a skeletal muscle biopsy or a placebo solution in and around the cardiac scar. The myoblast

injections did not improve echocardiogroahic heart function. In addition to its failure to improve cardiac function, the myoblast graft increased the number of post operative arrhythmic events (Menasche et al 2008).

Muscle- derived stem cells are readily available, easy to amplify and have a good survival rate after implantation. Myoblasts have the potential to enhance the heart's contractile function as they retain their skeletal muscle cell's contractibility. In humans, the risk vs. benefit ratio of myoblast implantation to increase cardiac function of injured hearts has yet to be determined and must further be investigated.

D. Cardiac Stem Cells

1. Identifying Cardiac Stem Cells

The question of whether there are stem cells derived from cardiac tissue has been thoroughly investigated. Until recently, it was commonly believed that the adult mammalian heart is lacking in the ability to generate new cardiomyocytes: cardiomyocytes do not proliferate. This view is supported in part by clinical observation as primary myocardial tumors are rarely observed in adults (Soonpa and Field 1998). However, recent efforts looking for evidence of cardiomyocyte turnover in the adult mammalian heart have led to varying results.

Studies by Beltrami and colleagues (2001) challenging the dogma that the adult heart is a postmitotic organ, suggest that the human adult heart may have the endogenous capacity to regenerate. By staining the human heart tissue with an antibody specific for Ki-67, a protein closely associated with the cell cycle and cell proliferation, they concluded that there is extensive cardiomyocyte proliferation during normal aging and that the number of cardiomyocyte proliferation is markedly up regulated in and after myocardial infarction. Compared with the number of Ki-67 cells in a series of control hearts from patients who died of noncardiac causes, the number of Ki-67 cells in hearts from patients that have experienced AMI was 84 times higher in regions that bordered the infarct zone, and 24 times higher in regions of the heart distant from the scar. Based on this data, Beltrami calculated myocyte mitotic indexes of .08 and .03 percent in zones adjacent to and distant from the infarcts and suggests that the billions of cardiomyocytes lost in a heart attack are replaced in about 18 days.

Like Beltrami, Bergmann et al (2009)**,** studied the rate of cardiomyocyte turnover in humans. In a search for using an effective method to study cell turnover, Bergmann used a novel approach utilizing a disturbing chapter in the history of nuclear weapons to track cardiomyocyte proliferation. Bergmann took advantage of Spalding et al's (2005) study describing that testing for the radioactive isotope Carbon 14 in cells can reveal the cells age. Bergmann's predecessor discusses how testing of nuclear weapons during the Cold War resulted in a dramatic global increase in the levels of the isotope Carbon 14 in the atmosphere, followed by an exponential decrease after 1963. His work indicates that Carbon 14 in genomic DNA closely parallels atmospheric levels and can be used to establish the time point when the DNA was synthesized and cells were born, thereby measuring the rate of cell turnover. With this effective cardiomyocyte dating method, Bergman' s group determined the age of cardiomyocytes in humans and discovered that cardiomyocytes do indeed renew over the human lifespan albeit at the very slow rate of 1% turning over annually at the age of 25, decreasing to .45% at the age of 75.

Bergman and Beltrami's findings indicate a large discrepancy in the rate of cardiac cell turnover. This may be due to the fact that Bertrami's method of reporting dividing cardiomyocytes with the Ki-67 antigen may have produced drastically exaggerated numbers of reproducing cardiomyocytes. The number of cells with the Ki-67 antigen may be representative of all cells in cardiac tissue in mitosis, not only the cardiomyocytes, and thus Beltrami obtained such a large number in his study on cardiac cells replication. Also, it has been pointed out by Soonpa and Field (1998) that Ki- 67 staining in the heart does not unequivocally establish that cell division has occurred, since a substantial amount of cardiomyocytes are binucleated or polyploidy and in the context of AMI, polyploidy may precede cell death and thus the elevated number of Ki-67 cells during infarction is accounted for.

Research by Kajstura et al (1998) indicates proof of myocyte proliferation. Using confocal microscopy, this investigative group reports that 14 myocytes per million were in mitosis in control human hearts, and a nearly 10- fold increase of this parameter was measured in end stage ischemic heart disease (152 myocytes per million). They also suggest that large numbers of myocytes can be formed with time as mitosis only lasts one hour. Urbanek et al's study (2003)on the myocyte formation from cardiac stem cells in human cardiac hypertrophy indicate that the human heart contains a population of cardiac stem cells that can divide and differentiate into myocytes and that this cardiac stem cell pool is enhanced acutely after infarction.

Although these findings are novel in that never before was mitosis seen in cardiac myocytes and that cardiac cells were believed to be incapable of dividing, according to one research group these reports don't provide much clinical use. Leri et al (2005) describes that the problem of compromised cardiac function following an ischemic event isn't the lack of myocytes, it is the accumulation of old poorly contracting cells and the extensive myocardial scarring that occurs following AMI. Leri describes that 12-72 hours following an ischemic induced myocardial necrosis there is an infiltration of neutrophils and an accumulation of macrophages which initiate the removal of necrotic myocardial tissue. Within seven days, scarring, that starts with the deposition of granulation tissue, is initiated and increases over a period of several weeks. It is this scarring that albeit protects the heart form aneurismal dilation contributes to reduced ventricular function in the most ischemic heart. In addition, the non contractile nature of the scar will lead to compensatory ventricular remodeling of nonischaemic myocardium and eventually heart failure.

Leri explains that contrary to the general belief that the heart's restricted regenerative ability is representative of the initial event of impaired cardiac function, it is the newly formed non contractile cells that are responsible for the impaired cardiac function post AMI. The inevitable scar formation that results from cardiac cell aplasia decreases the heart's contractile capacity. The need to surpass the fibrosis had led researchers to develop strategies to replace dead cells with viable cells. Bone marrow derived cells, adipose derived cells, skeletal myoblasts, cardiac cells and embryonic cells are being used experimentally to replace the rigid scar forming tissue in the heart as we are learning. The implanted stem cells most likely form a passive graft thereby decreasing the stiffness of the scarred portion of the ventricular and thus enhancing the heart's contractile function. According to Leri it is the elastic qualities of the new cells that contribute most to enhanced heart function.

2. Cardiac Stem Cells used for therapy

Adult cardiac stem cells(CSCs), isolated on the basis of various stem cells markers such as stem cell antigen c-Kit (Bearzi et al 2007 and Bertrami et al 2003) are believed to be involved in maintaining myocardial homeostasis throughout life but seem to be incapable of counteracting massive degenerative events such as AMI themselves. Application of ex vivo human cardiac stem cells seems to be a promising approach to bolster the hearts inherent repair capacity. Unlike other stem cells, cardiac stem cells should be more effective in making new myocardium as CSCs are programmed to create heart muscle and upon activation can rapidly engender parenchymal cells and coronary vessels possibly rescuing the failing heart (Quaini et al 2002, and Urbanek et al 2005).

Dawn et al (2005) injected CSCs into the coronary arteries of rats four hours after coronary occlusion. Echocardiographical analysis showed that the CSCs attenuated the increase in LV end diastolic dimensions and impairment in LV systolic performance at five weeks after infarction. The CSCs induced myocardial regeneration decreasing the infarct size by 29%. Dawn's study establishes CSC as a possible candidate for cardiac regeneration and indicates that the hearts own stem cells could be collected and stored for therapeutic purposes. Studies by Beltrami et al (2003), Oh et al (2003) and Matsuura et al (2004) support Dawn's findings and reveal that the injection of CSCs locally promotes myocardial regeneration after infarction in rats and mice. Bearzi et al (2007) similarly studied the effect of injecting human cardiac stem cells in the infarcted myocardium of immunodeficient mice and rats and found that the human CSC generate a chimeric heart, which contains human myocardium composed of myocytes , coronary resistance arterioles and capillaries. After isolating the ckit + CSC from eight patients and injecting them into the infarcted myocardium of rats, Bearzi found that the human cardiac cells were structurally and functionally integrated with the rodent myocardium and contributed to the enhanced performance of the infarcted heart.

Aside from transplanting CSCs into infarcted myocardium, some researchers have studied the possibility of activating resident stem cells in cardiac tissue to repair the infarcted heart. Linke et al (2005) studied the dog heart to see if it contains resident cardiac progenitor cells that can be stimulated by the Hepatocyte Growth Factor (HGF)-Insulin like Growth Factor 1 (IGF1) receptor system to regenerate myocardium after infarction. Linke proposed that cardiac stem cells can be activated by this growth factor signaling system. Linke based his clinical trial on a study that describes how HGF enhances vessel growth and favors cell- extracellular matrix interaction, which may be critical during myocardial regeneration. The study further indicates that stimulation on IGF1 receptors prevents cell death and induces differentiation of cardiomyocytes (Reiss et al 1996). Linke's findings were that the dog heart possess a reservoir for cardiac stem cells characterized by undifferentiated cells that are self renewing, and able to differentiate into other cell lineages. In accordance with the previous findings of other researchers, Linke's results are expected. More importantly however, Linke confirmed the idea that the cardiac stem cells posses an HGF and IGF 1 receptor system that when activated induces their migration, proliferation and survival. Linke's group injected HGF and IGF1 intramyocardially to stimulate resident cardiac progenitor cells after infarction. Indeed, the formation of myocytes and coronary vessels within the infarct was stimulated and the newly generated myocytes expressed nuclear and cytoplasmic proteins specific to cardiomyocytes.

With all these findings, we learn that the presence of cardiac stem cells in myocardium seems to be undeniable, yet the origins of the stem cells still remain debatable. The question of whether CSCs are generated within the myocardium or as Urbanek et al (2003) suggests are continuously being supplied to the heart by the bone marrow through the systemic circulation is being investigated, yet for the purpose of this review directed on the implications of stem cells in cardiac injury, this question will not be addressed.

E. Embryonic Stem Cells

These stem cells, derived from the inner mass of the blastocyst, posses unique properties. Embryonic stem cells (ESCs) can be grown in vitro and reproduce indefinitely in their undifferentiated state. They also maintain the property of multilineage commitment having the capacity to differentiate into cells originating from any of the three germ layers of the embryo. Because of their enormous potential, human ESCs have been obtained with the expectation for a future successful application of their broad therapeutic potential to patients. The uses for ESCs can be significant as adult stem cells have certain limitations. In addition to their limited proliferative capacity, the plasticity of adult stem cells has recently been challenged (Amit et al 2000). Recent studies by Terada et al (2002) and Ying et al (2002) indicate that the assumed capacity of transdifferentiation of adult stem cells into other lineages in vivo may simply be the fusing of implanted cells with existing cell types rather than a direct conversion into cardiomyocytes. The aforementioned limitations to adult stem cells and the inherent electrophysiological, structural and contractile properties of ESC derived cardiomyocytes make ESCs an ideal donor cell type for cardiac tissue regeneration.

Still, while ESCs seem to be the ideal cell type for implantation in the injured heart, the utilization of ESCs in clinical practice is hardly existent because their adult stem cell counterparts present fewer dangers than ESCs. The primary risk associated with the utilization of ESCs is its potential to form tumors, a dangerous side effect stemming from the ESC's capacity to proliferate indefinitely. Human ESCs cultured in suspension form spontaneous cellular aggregates called Embryoid Bodies. These tumors- like structures are found both in vitro and in vivo after ESC implantation (Reubinoff et al 2000, and Thomson et al 1998).

Because of the risks associated with ECS therapy, only several sporadic studies have employed these cells in their uncommitted state to repair the damaged heart. These reports, while indicating an improved ventricular function in the heart, also reveal dramatic side effects including the formation of benign tumors and the engrafting and colonizing of all organs with the possible development of neoplastic regions by the intravenously injected ESCs. In an attempt to avoid these serious complications associated with ESC implantation, researchers utilizing ESC, use methods to partially differentiate the ESCs in vitro before their implantation into the injured heart. This degree of cardiomyogenic commitment enhances the engraftment of cells in the myocardium and attenuates the probability of ESC acquiring undesired cell lineages thereby reducing the risk of tumor formation. To date most studies on the use of ESC exploit this method of differentiation.

Although due to certain ethical issues, there aren't any clinical studies on the implantation of human ESC in the human myocardium, other studies on the cardiomyocyte differentiation capacity of ESCs have been explored. Kehat et al (2001) cultivated human ESCs and plated them to form Embryoid Bodies. Kehat's group found that 8.1% of the cellular aggregates displayed contracting areas and the cells within these spontaneously contracting areas

stained positive for anti-cardiac myosin heavy chain, anti-cardiac troponin and various other cardiac specific features. Under electron microscopy, the areas also displayed varying degrees of myofibril organization consistent with early stage cardiomyocytes.

Clinical trials by Hodgson et al (2004), Min et al (2002) and Min et al (2003) study the effects of transplanting ESC derived cardiomyocytes into the hearts of rats. Hodgson injected infarcted rat hearts with ESCs preexamined for cardiogenicity. The trial found that stem cell delivery generated new cardiomyocytes that integrated into host myocardium and was associated with normalized ventricular architectures, little scar, and decrease in signs of myocardial necrosis. Hodgson's study points out that the ESCs injected were well accepted by the rats as no evidence of graft rejection, or tumor formation was observed. Hodgson's findings conclude that ESCs through differentiation within the host myocardium, can contribute to a stable beneficial outcome on contractile function and ventricular remodeling in the infarcted rat heart

Min et al (2001, injected ESCs into the infarcted hearts of rats intramyocardially. Compared with a control group injected with the equivalent volume of cell free medium, the experimental rat's cardiac function was significantly improved six weeks after cell transplantation. Min's study indicates that embryonic stem cells can be implanted and survive in injured rat myocardium and that these cells improve myocardial function. In a later analysis, Min et al (2003) studied the prolonged effects of ESC transplantation in the infarcted heart. His group injected rats with ESCs stained with GFP and studied the heart and its function 32 weeks after infarction and subsequent injection. After 32 weeks, tissue positive for GFP was found in the myocardium suggesting that the engrafted cells were still present. Echocardiographic data also showed that ESC transplantation significantly improved ventricular function relative to the control group even after four months.

In contrast to the findings of Hodgson and Min, Fijnvandraat's study on embryonic stem cells reveal that ESCs are not capable of restoring cardiac function in the infarcted heart. In 2003, FIjnvandraat in his study on in vitro differentiated embryonic stem cells and its subsequent cardiogenesis in vivo and in vitro found that ESC derived cardiomyocytes maintain a phenotype of the primary heart tube, and not of the chamber myocardium. Fijnvandraat, with very specific regulation of gene expression discovered that the ESC derived cardiomyocytes do not develop into fully mature chamber myocardium, and rather show a phenotype comparable to young embryonic cardiomyocytes in vivo and thus would not be of great importance in the area of restoring cardiac function(Fijnvandraat et al 2003).

The clinical implications of these findings seem somewhat promising as rat ESCs display the potential to restore damaged rat myocardium. Due to certain ethical concerns that lead to complications in obtaining human ESC it may seem plausible to use rat ESC to restore human myocardium. Yet Mcdevitt et al (2005) and Kehat et al (2001)'s studies display that rat and human ESC are very different and thus cannot be used interchangably. Mcdeviit studied proliferation patterns in human and mouse embryonic stem cell derived cardiomyocytes. The study revealed that cardiomyocytes differentiating from human ESCs exhibit a high level of proliferation which progressively decreases as the cells mature in culture. In stark contrast to the cardiomyocytes derived from human ESCs, cardiomyocytes derived from mouse ES cells appeared to be non proliferative using similar experimental techniques.

Kehat, in his work notes that there are some major differences in human and murine ES cell differentiation because there are significant differences in human and murine development.

Kehat compares his results in the differentiation of human ESCs with the rate of differentiation in mouse ESC. He explains that differentiation of human of ES cells proceed at a much slower rate than in mouse ESCs. In mouse cells, cells are grown in suspension for five days and spontaneously contracting areas appear one day after plating and within two- ten days 80-90% of EBs reveals pulsating areas. In contrast to this, human ESCs are grown in suspension for ten days and the spontaneous contracting areas did not appear until day four after plating; and the percentage of pulsating areas is a mere 8.1% Thus to have any really benefit for human myocardial regeneration clinical trials focusing specifically on human ESCs must be utilized .

Min et al (2002), in their experiment also spotted a group of growth factor proteins that seems to be involved in cardiac organogenesis in the early embryonic stages. They noted that different growth factors like vascular endothelial growth factor (VEGF) seem to enhance the functional improvement of post infarcted mouse hearts with transplantation of ESCs. To test whether specific growth factors would assist the ESCs in differentiating into functional cardiac tissue, Kofidis et al (2005) injected undifferentiated and labeled ESC into the infarcted mouse myocardium, added growth factors, and comparatively evaluated the restorative effect of their transplantation. Kofidis's group injected rat hearts with ESC supplemented with fibroblast growth factor (FGF), and ESC supplemented with transforming growth factor (TGF) and compared the subsequent cardiac function to a control group of rats injected only with the growth factors and not the ESC. Each group of growth factors had different effects on the capacity for regeneration. Kofidis found that TGF administration, compared with FGF and VEGF displayed the most restorative and differentiation potential. To further test the effect of supplementing growth factors with ESC, Yang et al (2002) studied VEGF to examine if an improvement in blood flow to ischemic regions can be found. Yang injected ESCs over expressing VEGF into the hearts of mice post infarction and compared the cardiac function to group of mice injected with normal ESCs. The effects of the ESCs and the ESC over expressing VEGF on neovascularization in ischemic were evaluated and compared. Improvement in left ventricular systolic function was significantly greater in post AMI mice transplanted with ESC plus VEGF. In addition, Yang found that the ESC themselves expressed certain amounts of VEGF and thus were able to stimulate the growth of new blood vessels in injured myocardium.

Earlier on, Kofidis et al (2004) administered a study on growth factors and explicitly studied the effects of Insulin- like growth factor -1 (IGF-1) in enhancing the proliferative capacity of ESC. His group implanted ECSs with IGF- 1 in an attempt to enhance the restorative capability of ESC on injured myocardium. They found that IGF -1 promoted expression of cardiomyocyte phenotype in ESCs in vivo. Their findings indicate that supplementation with IGF -1 enhances a- sarcomeric actin expression, and expression of MHC 1 thus indicating a stronger differentiation potential in the stem cells supplemented with IGF- 1. A trend in better myocardial fractional shortening in the group of mice treated with ESC and IGF-1 was also noted. These studies are significant as they suggest that growth factors may be supplemented with pluripotent cells to drive their differentiation to the desired phenotype and enhance their engraftment, leading to more efficient use of stem cells in cardiac therapy.

Kofidis further describes that contrary to the notion that embryonic stem cell are not susceptible to a host's immune response, ESC transplantation may face similar issues of allorecognition as solid organs transplantation. The idea that ESCs express only small amounts of MHC I, a cell marker foreign to cardiac tissue, in culture and thus are not susceptible to immunorejection (Kaufman and Thomspon 2002)is refuted in Kofidis's experiment as he found

nearly 70% of the injected ESCs in vivo expressing MHC I. In a series of immunological experiments in their lab, Kofidis's group obtained evidence of a humoral response in donor cells. They detected an increased production of Interferon- gamma, Interleukin -2 (IL-2), IL-4, and IL-5 by activated splenocytes of the host in response to ESC injection into the heart. Even so, many clinical trials including that of Hodgson's involving the injection of ESC in the hearts of rats in vivo have displayed no real signs of immunorejection.

Embryonic stem cells posses certain advantageous characteristics in their use for stem cell therapy. Their unlimited proliferative capacity, coupled with their ability to differentiate into any type of cell make them a model contestant for stem cell therapy. In contrast to their adult stem cell counterparts, studies with ESCs have yielded mostly unvarying results in their ability to differentiate into cardiac myocytes and their contribution to improved myocardial performance post myocardial infarction. The dangers associated with transplanting ESC can be circumvented by plating the ESC in vitro to start their cardiomyogenic differentiation before being implanted in vitro. Due to certain human ethical concerns and the subsequent lack of funding, current studies on the implantation of ESC and their functional benefit in damaged human myocardium are lacking. Hopefully this will soon change and therapeutic effects of these stem cells will be taken advantage of.

3. Conclusion

Cellular therapy is the latest in a series of strategies applied in an effort to mitigate the progressive and otherwise irreversible loss of cardiac function that frequently follows a heart attack. The need for an effective therapy to restore cardiac function following a cardiac event in which cardiomyocytes atrophy is essential. Heart failure, the condition that occurs when the heart's contractile reserve is depleted below a critical threshold, is already the most common cause of hospitalization in US citizens over 65, and, as our population ages, some have predicted epidemic proportions of this diseases (Laflamme and Murry 2005). The proposal that heart failure could be reversed or prevented if new myocardium could be grown in diseased hearts is excitedly being explored. This idea has gained widespread attention recently, leading to numerous reports and multiple early stage clinical trials in this field. In this review a summary of current data arising from clinical applications involving hematopoietic, adipose-derived, skeletal muscle-derived, cardiac and embryonic stem cells in cellular therapy for acute myocardial infarction is presented. While studies of cardiac regeneration utilizing these stem cells seem promising and are advancing at a quick rate, perfecting our interventions to repair the heart will take continuous effort and many years.

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Cardiac Regeneration

Sara Leah Abraham

Introduction

Cardiovascular disease is a generic term that refers to any illness or disorder that involves the heart and its vessels or the blood vessels of the body. Cardiovascular disease has been accepted as the leading cause of death worldwide. According to the Centers for Disease Control's National Vital Statistics Reports, twenty six percent of deaths in 2006, 631,636 in total, were caused by diseases of the heart (Heron et al. 2009).

One of the most common of all cardiovascular diseases is Ischaemic Heart Disease. This coronary artery disease often leads to Acute Myocardial Infarction, more commonly known as a heart attack. An ischemia occurs when an organ is receiving an insufficient supply of blood, often caused by a clogged artery (De Milto 2006). Atherosclerosis and blood clots in some of the larger coronary arteries are the most common condition to block coronary circulation. Coronary arteries take rise from the root of the aorta and spread out over the epicardium. These arteries branch out into energy hungry cardiac muscle, the myocardium, to supply it with oxygen and nutrients. The blockage of these deep arteries is known as myocardial ischemia. When ischemia to a specific region is severe, injury occurs (American Heart Association 2008). When blockage results in cell death, the condition is called an infarction.

Myocardial infarction is characterized by necrosis, cellular death in an isolated region of the heart. Cellular death stimulates the migration of macrophages to the infarct zone. They remove the necrotic tissue, and the area is refilled with a dense collagen scar, leading to a decrease in muscle thickness. The scar tissue, by and large, is acellular, and does not have the biochemical properties of myocardial cells. This can lead to electrical inconsistencies, loss of structural integrity, and mechanical dysfunction, such as arrhythmias. Together, these abnormalities can lead to complete heart failure (Joggerst and Hatzopoulos 2009).

Current treatments for post myocardial infarction patients aim to assist in the healing process and prevent further heart attacks, such as cholesterol-lowering medications and angiotensin inhibitors. A rehabilitation plan that includes blood pressure and cholesterol management may be set up. Diet and exercise regimes may change (Mayo Clinic 2010). Ultimately, though, once an area of the myocardium is dead, it's irreversible. It is because of this that scientists are looking for ways to alleviate the effects of myocardial ischemia and infarction by means of cardiac regeneration.

Cell Therapy

The basic concept of cell therapy is that exogenous, or possibly even autologous cells implanted into diseased tissue can recover and improve its function by substituting for the resident cells that have died. The improved neurologic function of Parkinson's patients that underwent the grafting of intracerebral dopaminergic cells and the prospect of weaning a number of diabetics off insulin after Langerhan islet transplants provide a good indication that cell therapy can be clinically effective. Transplanted cells must be engrafted in very specific quantities, and they must be able to integrate into normal host tissue without excess reproduction

and other adverse effects. When attempts to ameliorate the effects of myocardial infarction by converting the infarct zone into contractile tissue with the use of viral vectors encoded with muscle specific master genes failed, cell therapy became an increasingly attractive method of attempted cardiac regeneration (Menasche 2004).

Stem Cells:

Stem cells can be defined by two significant characteristics. First and foremost, they must be able to self-renew. They must be able to go through many cycles of mitosis and still remain in their undifferentiated state. Secondly, a stem cell must have the capability to differentiate into various specialized cell types. The effectiveness and value of stem cells are often based on their potential to do this. Pluripotent stem cells are able to differentiate into specialized mature cells from the three germ layers. The embryonic stem cell is the classic example of a pluripotent stem cell. Multipotent stem cells, alternatively, are limited to differentiating into mature cells of directly related lineages, generally from the same germ layer. Adult stem cells, sometimes called somatic stem cells, that are derived from postnatal, or even fully mature tissue are generally multipotent. Some adult stem cells sometimes have a more limited capacity for self-renewal, and are committed to differentiation into specific lineages. These cells are called adult progenitor cells (Cook et al. 2009). Until very recently, the myocardium has been labeled and viewed as an end-differentiated organ – one without any potential for regeneration. Certain types of stem cells are currently being tested as sources for regenerative potential for the heart. There is a new availability for hope in the possibility of reconstructing the infracted, failing ventricle (Loscalzo et al. 2008).

Mesenchymal Stem Cells:

Mesenchymal stem cells (MSCs) were first identified by their ability to form fibroblast like populations, and have been recognized for their plasticity. In the past, they have been known to help normal bone regenerate in vivo. They were initially discovered in the stroma of bone marrow. It has been established that by secreting growth factors important for proliferation and colony stimulating factors, MSCs provide support for hematopoiesis. While generally harvested from bone marrow, MSCs have recently been found in and isolated from the placenta, adipose tissue, the liver, and umbilical cord blood. MSCs, an integral part of the composition of endothelium (perivascular cells), are now seen as ubiquitous. No single, specific marker for MSCs has been discovered yet, though they are characteristically lacking typical hematopoietic antigens, such as CD 34, CD 44 an45, and more. MSCs produce a large number of cytokines and growth factors, including the important vascular endothelial growth factor . The hallmark and most distinctive feature of the mesenchymal stem cell is its inherent ability to differentiate into anything of mesodermal lineage, such as bone, cartilage, tendon, muscle, and adipose tissue. The main advantage of MSCs in cell therapy is their unique immunological properties. They can be transplanted across major histocompatibility barriers; recipients will not need to take immunosuppressants. They can be taken from any healthy donor, even an unrelated one, and cryopreserved until needed. With their ability to self-renew and differentiate, MSCs can be induced to differentiate into cardiomyocytes, and there is evidence that MSCs migrate primarily to sites of inflammation. These factors together suggest their potential for treatment for ischemic myocardial infarction. A disadvantage of MSCs is that they are perhaps too heterogenic, and

there are concerns that they may provide unexpected results, such as ossification (Cook et al. 2009).

Intracoronary injections of autologous bone marrow have led to moderate recovery of cardiac function, but clinical findings show that bone marrow cells don't actually engraft in the infarct zone, nor do they reduce infarct size. A placebo controlled study was therefore done to increase the evidence that MSCs have reparative properties. To ensure that mesenchymal stem cell grafts were allogeneic, donor pigs were of a different strain than recipient pigs. The bone marrow was obtained from the iliac crests of male swine. MSCs were identified and isolated by density, and were plated to expand in culture, and were then cryopreserved. They were magnetically stained in order to be later identified under MRI. Recipient pigs underwent a surgically induced myocardial infarction via balloon occlusion of the left anterior descending coronary artery for sixty minutes, followed by reperfusion; left ventricular performance and cardiac oxygen consumption was measured. After three days, animals were randomly chosen to receive intramyocardial infusions of allogeneic porcine MSCs or a placebo through a needle tipped injection catheter. Contractility of the myocardium was measured by the maximal rate of isovolumetric contraction and ventricular elastance, and the ratio of left ventricular systolic pressure to stroke dimension. At day three post injection, no animal had died or shown signs of arrhythmias, or shown signs of cardiac perforation, thus indicating that the method of delivery was safe. The animals were euthanized after eight weeks. Their hearts were analyzed in gross and microscopic levels. Tissue samples were taken from the infarct zone, the infarct border, and remote tissue. Immunostaining revealed the presence of α-actinin, troponin-T, and myosin heavy chains. Histologic evaluation demonstrated that MSCs were found present throughout the infarct and border regions, and they expressed muscle specific proteins that weren't found while they were still in culture plates. They were also found in vascular structures, where they had incorporated into vascular smooth muscle and endothelium. Myocardial infarction normally begins at the subendocardial area of the myocardium and moves out through the midmyocardium out towards the subepicardia

Table 1. Hemodynamic measurements

		8 weeks post-MI	
	Normal	Placebo	MSC
LV end-diastolic pressure, mm Hg	8.4 ± 2.3	29.8 ± 7.6	20 ± 6.4†
LV end-systolic pressure, mm Hg	107.1 ± 4.2	117.7 ± 22.2	113.8 ± 5.8
Arterial elastance, mm Hg/mm	14 ± 2	26.1 ± 8.7	17.1 ± 3
dP/dt_{max}, mm Hg/s	2,560 ± 266	1,720 ± 351	2,465 ± 574†
Ees, mm Hg/mm	16.3 ± 2.4	7.9 ± 1.2	17.1 ± 2†
τ, ms	36.2 ± 1.8	52.6 ± 11.6	34.2 ± 1.2†
SW, mm Hg/mm	771 ± 116.5	470.3 ± 86.9	654.4 ± 129.3†
MVO_2, J per beat	3.2 ± 0.9	12.9 ± 1.4‡	3.7 ± 1.8‡
SW/MVO_2	9.1 ± 1.6	2.9 ± 0.1‡	10 ± 5.6‡

MVO_2 indicates myocardial oxygen consumption and SW/MVO_2 myocardial efficiency.
*$P < 0.05$ vs. normal (pre-MI).
†$P < 0.05$ vs. placebo (2-way ANOVA).
‡Listed values were measured at 4 weeks.

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region. In animals that received a mesenchymal stem cell transplant, the infarct region was confined to the midmyocardium. When measured, the subendocardial rim was thicker in the mesenchymal stem cell-treated group than in control groups. MSCs probably caused this cardiac regeneration of the subendocardium. The effects of the transplant on cardiac function are as follows (see table 1).

The animals exhibited recovery to almost normal levels of both systolic and diastolic function. Myocardial efficiency increased, almost bringing stroke work back to a normal level. Cardiac energy metabolism was nearly recovered (Amado et al 2005).

Hematopoietic Progenitor Cells /Bone Marrow Mononuclear Cells:

Hematopoietic progenitor cells are the only stem or progenitor cell that are routine in clinical use today, due in part to the fact that they are easy to isolate and feasible to implant (Joggerst and Hatzopoulos 2009). They reside in bone marrow, and are responsible for making all blood cells, constantly repopulating the hematopoietic and immune systems. They are used in bone marrow transplants to treat many disorders, including leukemia and aplastic anemia. In humans, hematopoietic progenitor cells are identified by the CD 34 marker (a surface gylocoprotein). Hematopoietic progenitor cells home to bone marrow in healthy mammals, where they adhere firmly to the endothelium. Whether or not hematopoietic progenitor cells actively participate in the repair of cardiomyocytes after an infarction is a matter of debate, though they have been known to differentiate into skeletal muscle fibers. It is generally accepted, though, that hematopoietic progenitor cells are responsible for the inflammatory wound healing process (Cook et al. 2009). Experimental studies have suggested that intramyocardial or intravascular administration of bone marrow derived he (Amado et al. 2005) progenitor cells may play a part in the functional regeneration of infarcted c enhancing neovasculogenesis of an ischemic myocardium (Schachinger et al. 2006).

Patients, aged eighteen to eighty, were eligible for the REPAIR-AM Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction) trial if they h had an ST-elevation myocardial infarction with residual left ventricular wall motion abnormality and a significantly decreased left ventricular ejection fraction (Schachinger et al. 2006). (A normal resting ejection fraction, the fraction of blood pushed out of the heart by the ventricles per beat, is 62.3±6.1% [Pfisterer and Battler 1985].) This placebo controlled, double blind randomized trial was performed in seventeen different centers, at a median of 4 days after AMI reperfusion therapy. Bone marrow biopsies were done on 204 patients and the aspirate was sent to a single cell-processing laboratory, where patients were randomized to receive either an intravascular infusion of bone marrow derived hematopoietic progenitor cells (101 patients) or a placebo medium (103 patients). This infusion was done using a stop-flow technique via balloon positioned in the infarct-related coronary artery. After four months, the BMC (bone marrow cells, and alternate name for hematopoietic progenitor cells) group showed enhanced contractile recovery of left ventricular function. One year later, the results of the experiment were measured. A total of eight deaths occurred in the year, six in the placebo group and 2 in the BMC group. None of the patients in the BMC group experienced a second myocardial infarction, while six patients in the placebo group suffered a total of eight myocardial infarctions, six of which were located to the target blood vessel. While on paper these numbers may seem small, statistically, the difference is significant. Similarly, additional revascularization procedures were needed less frequently in the BMC group than in the placebo group. Thirty-

eight revascularizations were needed in thirty seven patients of the placebo group, while only twenty five were needed in twenty two patients of the BMC group (Schachinger et al. 2006).

The BMC group can be seen as a predictor of a reduced cardiovascular event rate. It is reassuring that in every endpoint, be it death, another myocardial infarction, or rehospitalization due to heart failure, there was a trend, albeit a statistically insignificant one, in favor of the BMC group. This data suggests that the contractile recovery seen at four months may possibly translate into a better clinical outcome one year post BMC infusion (Schachinger et al. 2006).

Cardiac Stem Cells:

The steps that have been made towards cardiac regeneration through the various stem cell types prompted further research into any natural regenerative properties and mechanisms that cardiac tissue might have. The heart has always been viewed as a postmitotic organ because mature cardiomyocytes don't reproduce or propagate. Contradictory facts seemed to accumulate as cardiomyocytes were discovered proliferating and reentering the cell cycle in certain pathological conditions such as ischemia and hypertension (Joggerst and Hatzopoulos 2009). After a female heart was transplanted into a man, male cardiomyocytes and endothelial cells were found growing within the heart. Y-chromosome positive cells must have migrated from the recipient's atrial stump or bone marrow into the donated heart, and differentiated into

Table 2
Clinical events during 1-year follow-up

Number of patients with events	Placebo[a] (*n*=103)		BMC (*n*=101)		*P*-value
	n	%	*n*	%	
Death	6	5.8	2	2.0	0.28[b]
Cardiac death	4	3.9	2	2.0	
Myocardial rupture	1	1.0	1	1.0	
Myocardial infarction	1	1.0	0		
Sudden death	1	1.0	1	1.0	
Heart failure	1	1.0	0		
Cardiovascular death (stroke)	1	1.0	0		
Non-cardiovascular death (cancer)	1	1.0	0		
Myocardial infarction	6	5.8	0		0.029[b]
Rehospitalization for heart failure	3	2.9	0		0.25[b]
Revascularization	37	36	22	22	0.026[c]
Target vessel revascularization	26	25	16	16	0.097[c]
Stent thrombosis	3	2.9	1	1.0	0.62[b]
Non-target vessel revascularization	16	16	7	6.9	0.052[c]
Documented ventricular arrhythmia or syncope	5	4.9	5	5.0	1.0[b]
Ventricular arrhythmia	4	3.9	5	5.0	0.75[b]
Syncope	1	1.0	0		1.0[b]
Stroke	1	1.0	1	1.0	1.0[b]
Cancer	2	1.9	0		0.50[b]
Combined events					
Combined death or myocardial infarction	10	9.7	2	2.0	0.019[c]
Combined death, infarction, or any revascularization	42	41	24	24	0.009[c]
Combined death, infarction, or infarct vessel revascularization	31	30	18	18	0.040[c]
Combined death, infarction, or rehospitalization for heart failure	12	12	2	2.0	0.006[c]

[a]In 3 patients, only the 4 months follow-up was available.

[b]Fishers exact test.

[c]Chi-square test.

(Schachinger et al. 2006)

functional cardiomyocytes (Quaini et al. 2002). Since then, several types of cells have been discovered in the adult heart that have stem cell characteristics. One stem cell characteristic is a cytoplasmic exclusion of certain vital dyes, like Hoechst 33342 and Rhodamine 123. Populations of these cells, sometimes called side population cells, have been found in various organs, such as skeletal muscle, bone marrow, and adipose tissue. These cells have recently been found in cardiac tissue as well, concentrated in the deep tissue of atria and apex. Cardiac side population cells have been seen to differentiate into cardiomyocytes, suggesting that they may in fact be cardiac progenitor cells. After cardiac injury, such as ischemia, these cardiac side population cells are mobilized and move to the injury site. The apparent conflict between the existence of cardiac progenitor cells and the heart's lack of regenerative ability is still puzzling to researchers, though two theories have been developed. The first is that these cells, along with mature cardiomyocytes, cannot survive the hypoxic conditions of ischemia. The second is that the pool of cardiac stem cells diminishes with age, which can possibly contribute to lack of regeneration in the elderly (Joggerst and Hatzopoulos 2009).

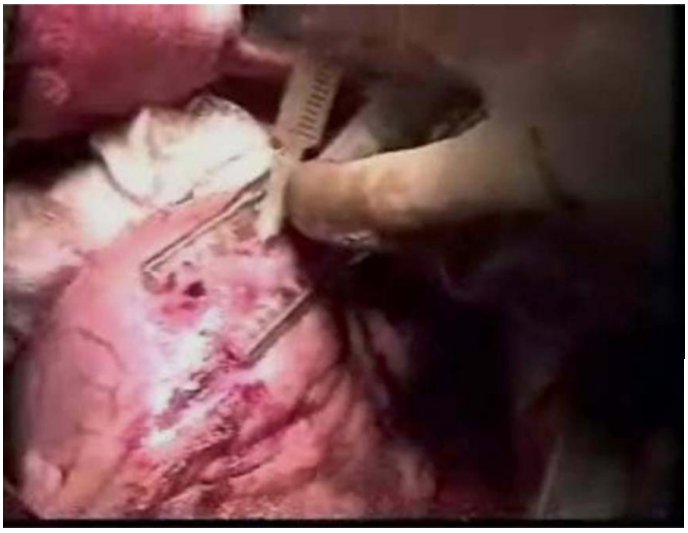
Studies have found what have been loosely termed human cardiac stem cells in small niches in the heart. These small clusters of human cardiac stem cells are closely connected to myocytes and fibroblasts by gap junctions and adherans junctions. The myocytes and fibroblasts form supporting walls that contain the cardiac stem cells. These cells are very obviously committed to myocyte lineage. Stem cell antigen C-kit^{POS}, sarcomeric proteins, and myocyte transcription factors are co-expressed consistently, though they sometimes also expressed transcription factors for endothelial cells and smooth muscle cells. Myocardial samples were enzymatically dissociated, and C-kit^{POS} cells sorted out and plated; multicellular clones were formed successfully in eight out of twelve cases. These clones were, in turn, plated again, or placed in individual wells. Doubling time was approximated at 29 hours. These cells differentiated into cardiomyocytes, smooth muscle cells, and endothelial cells. Developing myocytes had sarcomeric units, were striated, and showed contractile activity after electrical stimulation (Bearzi and Rota 2007).

Human cardiac stem cells were collected from eight patients and injected into infarcted mouse or rat heart, forming chimeric organs that contained human myocytes and coronary vessels. Cell therapy led to regeneration in the infarct zone. The new cells tested positive for α-sarcomeric actin and human DNA sequences were found in seventeen out of twenty five treated mice and fourteen out of nineteen rats. Of the new tissue, ≈84% was myocardial and ≈8% consisted of new microvasculature, making an approximate ration of one capillary to eight cardiomyocytes. Two different lentiviruses were injected into the cardiac stem cells and the infarcted rat and mice hearts to promote different dye production, and the results showed that there had been no cell fusion. The new cardiomyocytes were all human, and that animal cardiomyocytes did not contribute to the regeneration or repair. An examination of ECG done on injected hearts showed that tissue regeneration had partially restored contractile function in the infarct, resulting in an increased ejection fraction and a general improvement of ventricular function. When examined, the synchronicity of calcium tracings in both human and rat cardiomyocytes proved their functional integration. An occasional protein was even found linking human and rodent cardiomyocytes. Together, these observations all prove the role of transplanted human cardiac stem cells in cardiac homeostasis and myocardial regeneration (Bearzi and Rota 2007).

Skeletal Myoblasts:

While the regenerative ability of cardiac muscle cells is a relatively new development, it has been known for a while that skeletal myoblasts retain the potential to regenerate. This is due in large part to the presence of stem cells, sometimes called satellite cells, in this case, because of their location in the periphery of mature, multinucleated muscle tubules. A billion or more myoblasts can be easily cultured in the laboratory from a single small piece of muscle tissue. Myoblast therapy was developed originally as a possible treatment for muscular dystrophy, because myoblasts from a normal donor would fuse with the muscle of the recipient to provide normal proteins absent from dystrophic patient. In 1992, scientists realized the potential use of skeletal myoblasts in cardiac regeneration. Over time, experiments proved that myoblast transplantation could repair a damaged myocardium when cells engraft and integrate, adding new contractile muscle to the heart, and by promoting cardiac repair, including rejuvenation of blood supply, and therefore oxygen and nutrients, to previously damaged areas (Dinsmore and Nabil 2006).

Of one the wonderful things about myoblast therapy is that it can be autologus. In preparation for transplant, a skeletal muscle biopsy about five grams is done. The muscle is trimmed of all connective tissue and minced. It then goes through several cycles of digestion with trypsin and collagenase. The resulting cells are plated and grown in a medium developed primarily for supporting myoblast growth. The actual number of cells used for transplant can be anywhere from three hundred million to eight hundred million. Animal studies done with transplantation of skeletal myoblasts into ischemically damaged myocardium have demonstrated good results. They show that skeletal myoblasts engraft and contribute to improved heart function; they have survived long term without manifesting adverse affects, such as arrhythmias. Autologus rat myoblasts grafted into ischemic myocardium survived both inside and out of the infarct zone, and fused, forming myotubes in close contact with myocytes at the border of the infarct zone. Myocardial contractility and cardiac output increased in comparison to the control group. Ventricular volume and remodeling were minimal. In sheep, progressive heart failure halted short term , and was functionally reversed in the long term. Skeletal muscle fibers formed organized bundles that co-aligned with adjoining cardiac muscle. Muscle density in areas of ischemic collagen scars was almost restored to normal. Clinical studies have been done in both the USA and Europe, and direct evidence for skeletal myoblast survival had been provided. Autologus myoblasts formed myofibers that survived in the human myocardium and aligned



Autologous human myoblast transplantation in a human patient as an adjunct to coronary artery bypass surgery on a beating heart (Haider et al. 2004).

parallel to host cardiac muscle fibers, showing potential for synchronized contraction. This in turn, can contribute to improvements in systolic and diastolic functioning. Left ventricular ejection fraction increased. Tissue viability scanning of the scarred region of the myocardium six months post transplant showed improved metabolic activity (Dinsmore and Nabil 2006). While the theory shows great promise, a few problems with myoblast transplantation were exhibited in some trials. Ten patients that received myoblast transplants reported severe left ventricular dysfunction. Their ejection fraction dropped significantly to below 35%. In the MAGIC (Myoblast Autologuous Grafting in Ischemic Cardiomyopathy) trial, four out of sixty four patients developed ventricular tachycardia after receiving skeletal myoblast injections in coronary artery bypass grafting, and twenty four more were expected to. Therefore, clinicians

must proceed with caution in regard to arrhythmias in pursuing myoblast therapy. Furthermore, many myocardial infarction patients are not surgical candidates, and a less invasive method of delivery must be devised (Dinsmore and Nabil 2006).

Of all the various studies done to investigate the effects of cell therapy on myocardial infarction, few have taken timing into account, and as of yet, the optimal time for cell transplantation in heart attack patients remains unclear. One such study was done in 2009 using human umbilical cord blood cells (HUCBC), which are rich in mesenchymal progenitor cells and contain endothelial cell precursors with huge in vitro proliferation capabilities. Myocardial infarction was identified in eighty male Wistar rats by elevated ST segments in a transthoracic ECG. Equal volumes of either HUCBCs or a phosphate buffered saline placebo were injected into the rats through a caudal vein at days one, five, ten, and thirty after myocardial infarction. The rats were all sacrificed four weeks post infusion, after a hemodynamic assessment and an ECG. Mortality in rats transfused on day one post infarction was 40%, most deaths occurring within one week. Rats infused on day five and day ten both hard mortality rates of 10%, and those injected thirty days after the myocardial infarction had a mortality rate of 30%, usually at three weeks post infarction. Before injection, all rats had a similar left ventricular ejection fraction of about 45%. After four weeks, the left ventricular ejection fraction in five-day transplantation group had moved up to about 50% and the ten-day group reached almost 60%, doing significantly better than the control groups. Left ventricle wall thickening also improved significantly in the 10-day group, as opposed to the others. While scar formation was extremely noticeable in hearts injected with buffered solution, scar size was smaller in groups that received the HUCBC injection, more so in the five and ten day groups than the others. When injected cells were immunostained with anti-human leukocyte antigen, it was observed that only cells delivered in the ten-day group had settled in the infarct zone in the myocardium enough to make a difference. New angiogensis was due to the endothelial progenitors found in HUCBC. Microvasculature density was significantly greater in the five-day and ten-day groups than in their controls, and was altogether greater in the ten-day group. Myocardial infarction is a time dependent process, based on the loss of effective cardiomyocytes and the formation of scar tissue. The process begins with an inflammation of the infarct zone, and this reaction is nearly complete at ten days post myocardial infarction. It can be concluded that this is the best environment for stem cell homing and survival (Xing, Yun-li et al. 2009).

Tissue Engineering

Cardiac muscle tissue has a very quick and active metabolism. It therefore requires a lot of oxygen and nutrition. In natural cardiac tissue, almost every cardiomyocyte can be found in close proximity to a capillary vessel, ensuring that each cell is properly perfused and taken care of. Cardiac cell density is much higher than most other tissues. In a normal heart, non-cardiomyocyte cells such as vascular endothelial cells, smooth muscle cells, and fibroblasts make up 70% of the tissue. These non-cardiac cells are integral to normal heart functioning. All these factors together make it very difficult to artificially create cardiac muscle (Guo et al. 2009).

One study investigated the effects of co-seeding rat mesenchymal stem cells with embryonic cardiomyocytes. The scientists conducting the study believed that the only way to attempt to engineer cardiac muscle is in three dimensions, so they constructed a three dimensional tubular scaffold called a myotubule. They fed liquid collagen between two rotating

cones, resulting in a cylindrical tube of collagen fibrils with a hollow, inner central lumen. To isolate the mesenchymal stem cells, they anesthetized 300 rats and removed the femoral and tibial bones. The marrow cavities were flushed and the marrow was combined. The bone marrow was passed through thin needles to break up clumps, and the single cell suspensions that resulted were centrifuged for five minutes at 200g. Cells were trypsinized three times, and replated. Only bone marrow stromal cells remained. Cells were labeled with green fluorescent protein for lineage tracing, and were replated for subculturing. Embryonic cardiac myocytes were collected from rat embryos. Upon the dissection of their hearts, the atria were discarded. The ventricles were minced and incubated, and centrifuged, until ventricular primary cells could be isolated. The embryonic ventricular cardiomyocytes (ECMs) were planted in a medium filled collagen tube alone, and a combination of ECMs were seeded together with mesenchymal stem cells. The results of both experiments were studied at seven, fourteen, twenty one, and twenty eight days. Gene expression of the tissue in both myotubules was studied in order to analyze cardiogenic differentiation. In order to validate findings of cardiac myocyte markers in these collagen tube cultures, cardiac specific markers were immunocytochemically stained. This was done using antibodies directed against specific cardiac transcription factors, hormones, contractile and structural filaments, and junctional proteins. Through staining and lasers, it was established that the cells located in the myotubes tested positive for many cardiomyocyte markers, including myosin heavy chains, sarcomeric myosin heavy chains (a contractile protein), cardiac troponin, cardiac actinin and desmin, GATA binding proteins, peptide hormones ANP and BNP, and others (Valarmathi et al. 2010).

At 21 days, ECM tube cultures showed the presence of myocytes mainly on the luminal and outer surfaces of the construct. External to the tubule, ECMs aligned and overlapped in an orderly manner, but cells on the inner luminal surface showed cord like cellular arrangement that resembles that of in-vivo myocytes. These cells showed signs of developing sarcomeric units, and tested positive for actin and myosin. They indicated progressive differentiation towards in-vivo neonatal-like ventricular cardiac muscle cells. In MSC/ ECM co-cultures, the differentiating cells appeared organized into many layers of intercalated bundles and branches throughout the myotubule. Cross bridges and specialized cell junctions were evident. The maturing cardiomyocytes showed evidence of evolving into Z-disks and into cardiac specific sarcomeric arrangements, and showed promise for cardiac biosynthetic activities. The MSCs that had been immunostained showed markers associated with cardiomyocytes, including myosin heavy chains and other aforementioned characteristic, proving that it wasn't only the ECMs affecting the expression of cardiac properties. In comparison to the ECM only construct, hormone secretion levels of the MSCs/ECMs stayed strong and constant. The cells were metabolically active. When tested under electron microscope, the nuclei of ECMs were typical of underdeveloped embryonic cardiac muscle cells. The MSCs/ECMs co-culture revealed the typical appearance of developing cardiomyocytes. Cells were elongated, multilayered, and orderly. Myofilaments were present, if randomly dispersed throughout. Plus, the cells showed developing mitochondria and vescicles of the active cardiomyocyte. MSCs/ECMs combination expressed cardiac specific genes, proteins, ion channels, receptors. In addition spontaneous, synchronized contraction of the culture was evident through the transparent myotubule (Valarmathi et al. 2010).

Previous successes in the implantation of engineered cardiac tissue have failed due to necrosis at the core of the transplanted tissue and poor survival related to ischemic injury.

Bioengineers have long sought a solution to this problem. Human embryonic stem cells were differentiated into cardiomyocytes and suspended in a rotating orbital shaker, resulting in human cardiac tissue patches, composed of enriched cardiomyocytes. These patches, however, did not survive transplantation in-vivo. Scientists tried heat shocking the patches a day before implantation and bathing them in prosurvival cocktail before implanting them into skeletal muscle of nude rats. At one week, they found only rare, isolated human cardiac muscle cells. The peripheral edges of the tissue were viable, but the entire inner core was dead. They deduced that the problem was in large part due to the ischemic injury at the infarct zone, and that the patches were too thick for nutrients to diffuse into the core, and that in order for the procedure to be successful, the tissue needed to be vascularized. The tissue patches died before host angiogenesis could provide them with a normal blood supply (Stevens et al. 2009).

Tri-cell cardiac patches were scientists' next attempt. Human embryonic stem cell derived cardiomyocytes were combined with human umbilical vein endothelial cells (HUVEC) and mouse embryonic fibroblasts in a 1:1:0.5 ratio in a medium of human embryoid bodies (a cluster of heterogeneous embryonic stem cells that are set to differentiate to a specific lineage [Itskovitz-Eldor et al. 1999]). The newly created tissue had endothelial cell networks that resembled a vascular plexus. The next logical step was to test the contractility of the new tissue. Patches were stimulated using square waves of frequency and contraction was monitored via video edge detection. Cardio-HUVEC-MEF patches were cultured for 2-3 days before testing. They routinely contracted when stimulated by 2 Hz of electricity (120 beats/minute), but couldn't keep pace with 5 Hz of stimulation. When stimulated at higher frequencies, the patches never fully relaxed, and a decrease in contractile ability resulted. Another important consideration for scientists to take into account are the passive mechanical properties of cardiac tissue, especially in relation to diastolic filling. The passive stiffness of Cardio-HUVEC-MEF patches were tested in comparison to cardio only patches by using strips cut from each patch. They were stretched in increasing length increments. At 7.9 mN/mm^2, cardio-HUVEC-MEF constructs were closer to the stiffness of neonatal pig myocardium than cardio-only patches. When tested, scientists saw that the cardio-HUVEC-MEF constructs produced more connective tissue and collagen fibrils per area, making their stiffness more physiologically appropriate. These patches were a thousand times less stiff than the collagen scar that forms as a result of myocardial infarction, suggesting that they would impede much less on diastolic filling (Stevens et al 2009).

The next step for scientists was to test whether or not these vascularized human cardiac tissue patches could survive implantion in vivo. Prior to their implantation into the gluteus muscle of nude rats, cardio-only and cardio-HUVEC-MEF patches were heat shocked and bathed in pro-survival cocktail. At one week post implantation, the rats were killed. Human cardiomyocytes and endothelial cells were indentified through the immunohistochemical staining of β-myosin heavy chain and human complement proteins. In cardio-only patches, only the occasional, isolated human cardiomyocytes were detected. Comparably, cardio-HUVEC-MEF patches formed much larger grafts of human myocardial tissue. The cardiac muscle cells showed small, sarcomeric arrangements. Many human endothelial cells could be found among the patches, some of them even containing traces of red blood cells. Despite the fact that cardio-only patches were produced from 50% more cardiomyocytes, implanted cardio-HUVEC-MEF patches grew to be 11 times larger. Next, the cardio-HUVEC-MEF constructs were sutured into the hearts of nude rats. The patches had attached to the hearts of all test subjects. The microvessels

that had formed contained red blood cells and white blood cells, indicated that the new vessels had anastomosed with the normal rat blood vessels, creating a much more ideal environment for tissue survival. (Stevens et al. 2009).

In the ten years that scientists have been experimenting with tissue engineering, much progress had been made. Nevertheless, there are still many obstacles that need to be overcome before newly synthesized cardiac tissue can be used to alleviate the effects of myocardial infarction. One of the largest problems facing bioengineers is constructing tissue the proper size and shape to fit into human myocardium. So far, no three dimensional tissue construct has been thick enough or large enough. Three to four cardiomyocyte monolayers have been successfully stacked one on top of the other, but the thickness hasn't come close to that of human cardiac tissue. As for shape, researchers have been able to construct tissue in rings, strips, and squares. While each of those shapes has its own advantage, none of them come close to the shape of a natural human heart. Of course, another large issue is immunorejection. None of the methods developed thus far have used or yielded autologus material. All implantation studies have used immunosuppressants. Another commonly overlooked problem is that most media supplements used to grow the tissue are xenogenic, resulting in several issues including infection. New, non-xenogenic growth media must be developed before engineered tissue can successfully be transplanted into humans (Xing, Yu-jie et al. 2009 b). Until this tissue has been successfully transplanted, studies cannot be done to test how this tissue affects the functionality of the working heart.

Conclusion

Because myocardial infarction and the death of cardiac muscle has always been seen as irreversible, researchers have been working on many different ways to reverse the process and regenerate cardiac muscle. In most studies, heart function was partially, if not mostly restored, even if cardiac muscle itself was not actually produced. The reperfusion of existing muscle and the prevention of scar formation definitely played a part in reestablishing cardiac function and preventing further heart complications. Researchers discovered the existence of cardiac progenitor cells and have begun to experiment with them. Tissue has been engineered and successfully transplanted into rats. Much progress has been made in the field, though there are still obstacles that need to be overcome. Many studies featured embryonic cells, the use of which is still extremely controversial, and may lead to tumor formation in the long run (Joggerst and Hatzopoulos 2009). Finally, more human trials need to be done to prove the safety and benefits of cell therapy and tissue transplantation.

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Benefits of Breastfeeding

Renee Chill

Abstract-

When a child is born, a mother can choose to breastfeed her infant or to use an alternative source of nutrition such as formula. To choose properly, the mother must be informed about the pros and cons of each method of feeding. This paper will elaborate on the process of breastfeeding and present some of the benefits that are conferred to both infant and mother through the act of breastfeeding, benefits that formula does not provide. Breastfeeding positively affects infants in both the short- and long-term by providing protection from infectious diseases, fostering the psychological bond with their mothers, and increasing cognitive development later in life. In addition, mothers benefit from choosing to breastfeed their infants by having a quicker delivery of the placenta, retaining less weight postpartum, and having a decreased risk of breast cancer. In conclusion, this paper will show that while formula does contain essential nutrients for infants, it does not provide the protective and curative benefits that breast milk does.

Introduction-

Lactation is a fundamental part of the reproductive cycle of all mammals, including humans. After intrauterine and parenteral nourishment has terminated, and before individuals can independently sustain themselves, mothers can naturally provide their newly born children with necessary nutrition through breastfeeding. In the early twentieth century, statistics reveal a decrease in the percentage of infants who were breastfed, most likely due to a lack of knowledge of the health benefits that are associated with breastfeeding. However, as science continues to improve and more studies are conducted, it is becoming increasingly clear that human milk is the ideal source of nutrition for infants. Early scientific literature, generally from countries other than the United States, claimed that breastfeeding was better than bottle-feeding, as evidenced in mortality charts comparing the risk of death in both groups, but no reasons could be given to explain this assertion (Grulee et al., 1934,1935). Refinements in the analysis of food constituents have allowed for further studies of breast milk, revealing how it is physiologically suited for newborns. Developments in the study of infections have also identified the anti-infectious properties of breast milk, offering protection for the infant until weaning. Still other studies acknowledge various other health benefits of breastfeeding to both mother and infant. Recent statistics show an increased trend toward breastfeeding in the Western world, due to increased education of the benefits of human lactation. The objective of this paper is to show why breast milk is better for infants than alternative sources of nutrition, whether milk from animals or formula.

Discussion-

Anatomy and Physiology of breastfeeding-

Mammary glands begin to develop embryologically at six weeks, maintaining growth until milk ducts are developed by the time of birth. At puberty of a female, the breasts expand to their adult size, the left usually being slightly bigger than the right. During lactation, the breast weighs between 600 and 800 grams, compared to the roughly 200 grams in a nonpregnant woman and 400-600 grams in a pregnant woman (Lawrence, 1989).

The breast is made up of many different independent glands. The stroma and the parenchyma are two divisions that make up the corpus mammae. The stroma includes the connective tissue, fat tissue, blood vessels, nerves, and lymphatics. The parenchyma is comprised of the alveolar gland with ductular branching alveoli. Each gland opens into a lactiferous duct, whose distal end is dilated to form the lactiferous sinus. There is a slight constriction before opening onto the surface of the nipple (Lawrence, 1989).

The nipple, or papilla mammae, contains 15 to 25 lactiferous ducts, as well as smooth muscle fibers, sensory nerve endings, and sweat glands. The areola, which surrounds the nipple, contains Montgomery glands that become enlarged during pregnancy and lactation. They secrete a substance that lubricates and protects the nipples and areolae during lactation (Lawrence, 1989).

The lactating mammary gland is characterized by a large number of alveoli. Its functioning depends on the interplay of numerous intricate nervous and endocrine factors. Some are involved in mammogenesis, preparing the glands for lactation; others in lactogenesis, and others are responsible for the maintenance of lactation, or galactopoeisis. During pregnancy, a complex sequence of events, involving the hormone prolactin being synthesized by the adenohypophysis and released into circulation, as well as other hormones, prepares the breast for lactation. At delivery, with the ejection of the placenta, there is an abrupt decline in estrogens and progesterone. Since estrogens have an inhibiting effect on the secretion of milk by prolactin, their withdrawal triggers the onset of lactation. Suckling stimulates the release of prolactin, as well as oxytocin from the neurohypophysis. Prolactin stimulates milk synthesis, while oxytocin is involved in milk secretion (Mader, 2000).

Human breast milk is comprised of many different molecules, such as carbohydrates, proteins, and lipids, as well as minerals and vitamins. However, the composition varies with time of day, stage of lactation, maternal nutrition, and several other factors. Colostrum, the yellowish, thick fluid produced in the first few days following delivery, has a higher protein and lower fat content than mature milk, but energy and carbohydrate content are similar in both. It is rich in fat-soluble vitamin A, carotenoids, and vitamin E. This distribution reflects the needs and reserves of the newborn child (Sarkar, 2004). From approximately 7 -10 days to two weeks postpartum, the content of breast milk gradually changes to become mature milk. Mature milk is mainly made of water; all other constituents are dissolved or suspended in the water. Because 25% of the newborn's heat loss is from evaporation of water from the skin and lungs, water from breast milk helps regulate the infant's body temperature. Lipids represent the second largest percentage of the total breast milk composition. Proteins constitute about .9% of breast milk contents, with eight out of the twenty amino acids present being essential. The most prevalent carbohydrate in breast milk is lactose, or milk sugar, which is synthesized by the mammary gland. A number of other carbohydrates are present in the milk as well. Vitamins, minerals, enzymes, and hormones make up the rest of the components of breast milk (Lawrence, 1989).

Short Term Health Benefits to Infant-

The World Health Organization (WHO) recommends exclusive breastfeeding until the infant is six months of age because it protects against infectious morbidity, mortality, and promotes adequate growth and development (Kramer and Kakuma, 2001). The most important short-term immunological benefit of breastfeeding is the protection against infectious diseases. When the infant is first born, the immune system is in its nascent stage, placing the child at risk of infection. Colostrum, the specialized milk produced for the first few days following delivery, is much richer in immunoglobulins, antimicrobial peptides, and growth factors than mature milk (Playford, 2001). These components allow colostrum to be an important immune modulator, inducing maturation and differentiation of thymocytes, promoting peripheral blood leukocyte proliferation, and inducing cytokine production (Boldogh et al., 2008). Boldogh et al. (2008) conducted a study to investigate whether colostrum causes allergies and what its impact is on allergic sensitivity. The researchers found that colostrum does not increase IgE or IgG levels, which are the antibodies that cause the majority of allergic reactions. They also found that colostrum significantly decreased IgE and IgG production, airway eosinophilia, mucin production, and hypersensitivity to common allergens. These findings show the importance of feeding colostrum to newborn babies, as it is the best form of nutrition for the immature immune system. No other animal's milk or formula can provide for the precise physiological needs of the newborn human as well.

Mature human milk also contains numerous immune-related compounds, including oligosaccharides, cytokines, and interferons. Several of these components of milk offer passive protection in the upper respiratory system and gastrointestinal tract, preventing adherence of pathogens to the mucosa, and thereby protect the infant against invasive infections (Schack-Neilson and Michaelsen, 2007). One liter of human milk contains about five to ten grams of unbound oligosaccharides, which represents a major component of human milk, exceeding the amount of lipids. More than 130 different human milk oligosaccharides have been identified, both neutral and acidic, as well as combinations of the two. Compared with human milk, the concentration of oligosaccharides in the milk of domestic animals that is usually fed to infants is smaller by a factor of ten to a hundred. Infant formula contains only trace amounts of less complex oligosaccharides (Bode, 2006). The neutral fraction of human milk oligosaccharides appears to be the most relevant factor for the development of the intestinal flora typical for breastfed infants (Boehm and Stahl, 2007). Human milk oligosaccharides withstand the low pH of the stomach and resist degradation by enzymes from the pancreas and brush border membrane. Because of this resistance, these oligosaccharides are able to rinse the infant's esophagus, stomach, and small intestine, finally serving as nutrients for colon bacteria (Bode, 2006).

Most pathogenic microorganisms (e.g. *Campylobacter jejuni, Escherichia coli, Vibrio cholera,* and strains of *Shigella* and *Salmonella*) are only virulent with adhesion to the host's epithelial surface. Acidic oligosaccharides have been found to play an important role in the prevention of adhesion of these bacteria on the intestinal epithelial surface (Boehm and Stahl, 2007). Human milk oligosaccharides rinse the laryngopharyngeal region and may also reduce pathogen adhesion at the entry to the upper respiratory tract. Adhesion of *Streptococcus pneumonia* and *Hemophilus influenza* to pharyngeal epithelial cells, the most common cause of oritis media and respiratory tract infections in infants, is also inhibited by human milk (Bode, 2006). Acidic oligosaccharides are also involved in immune reactions, such as interacting with selectins in inflammation processes. Sialization of oligosaccharides can interfere with binding of

certain selectins and affect important regulators of the immune system (Boehm and Stahl, 2007). These effects of human milk oligosaccharides combine to provide extremely effective protection against intestinal infection and postnatal stimulation of the immune system. Because the quantity and diversity of oligosaccharides in human milk are distinct compared to those of other species, the use of other forms of nutrition aside from breast milk cannot offer the same oligosaccharide absorption, metabolism, function, and beneficial effects.

As stated previously, exclusive breastfeeding protects against gastrointestinal morbidity (Schack-Nielsen and Michaelsen, 2007). However, because iron concentration in human milk is low, breastfeeding may increase the risk for iron deficiency in some infants. Although iron-fortified formula may provide the necessary iron, the infant will be placed at risk for gastrointestinal infection. This is the case especially for infants of low-income households and communities where hygienic preparation of foods may be compromised, leading to more gastrointestinal infections and malnutrition. Monterrosa et al. (2008) conducted a study of infants born to low-income women in Guadalajara, Mexico. The researchers sought to investigate whether infants in this setting who were predominantly breastfed through the first six months of life would experience fewer gastrointestinal infections but be more likely to be iron deficient compared with infants who were fed iron-fortified formula on a regular basis. One hundred and fifty four mother-child pairs were recruited from the Hospital Civil Dr. Juan Menchaca, a certified Baby Friendly Hospital, where most of the patients are low income. Baseline data were collected while the mother and infant pairs were in the hospital, and follow-up visits on the infants' monthly birthdays were conducted as well. The study found that the non-predominantly breastfed infants (55 partially breastfed and 50 exclusively formula-fed) were almost twice as likely to have a gastrointestinal infection compared with the 49 predominantly breastfed infants. Mean iron concentration, however, was lower in the predominantly breastfed infants than in partially breastfed and formula-fed infants. The researchers concluded that even with the risk of iron deficiency, it is better for mothers to primarily breastfeed their infants until age six months. Iron deficiencies can be prevented by iron supplementation in pregnancy and by feeding the infant iron-rich foods at six months of age, but gastrointestinal infections can cause morbidity and mortality in infants (Monterrosa et al., 2008).

Another important aspect of breastfeeding is the close psychological relationship it fosters between mother and child because of the increased bonding opportunities. Research shows that breastfeeding mothers touch their infants more often both during feeding and while playing together. Mothers who breastfeed commonly exhibit more physiological and social responsiveness toward their infants. Infants who are breastfed are also generally more alert and responsive (Jones et al., 2004). Many women experience some kind of emotional imbalance in the first week after delivery, due to the enormous change in hormonal levels after the delivery of the placenta. However, some women become clinically depressed, suffering from post-partum depression. Depression can occur in all women, regardless of whether they are breastfeeding or not (Lawrence, 1989). Newborns of depressed mothers are found to demonstrate less left frontal brain activity, lower heart rate variability, and abnormal biochemical patterns, such as lower levels of dopamine and serotonin and higher levels of norepinephrine and cortisol, compared to newborns of mothers who are not depressed (Jones et al., 2004). One study (Jones et al., 2004) suggests that breastfeeding may protect newborns against some of the negative effects of maternal depression. The researchers compared infants of depressed mothers who were predominantly breastfed to those who were fully formula-fed by three months of age. In a

measurement of electroencephalographic activity, the breastfed infants displayed the same left frontal brain activity patterns as those demonstrated by infants of non-depressed mothers. Conversely, the formula-fed infants of depressed mothers showed less left frontal brain activity, indicating deficits in approach motivation. The study thus effectively demonstrates that "a stable breastfeeding relationship protects infants from some of the negative psychological and physiological effects in an environment of maternal depression" (Jones et al., 2004). Expectedly, though, depressed mothers are less likely to breastfeed and they breastfeed for shorter periods of time than non-depressed mothers. It is possible that if depressed mothers were to be informed of the significant benefits of breastfeeding and its protective factors for their infants, they would choose to continue breastfeeding.

Long-Term Health Benefits to Infant-

Aside from offering short-term protection from several diseases and infections, studies show that breast milk influences the child's own immune system. Infants are born with their immune systems not fully developed, dominated by subtype-2 helper T cells. Breastfeeding, through human milk oligosaccharides, speeds up the maturation of the immune system by stimulating the development of subtype-1 helper T cells. In fact, ultrasound measures of the thymus, an important structure necessary for T cell development and maturation, reveals that the thymus of breastfed infants at four months of age is twice the size of that in formula-fed infants (Schack-Nielsen and Michaelsen, 2007). Because breast milk contains immunomodulatory properties, breastfeeding has been found to protect against the later development of many immune-mediated diseases such as bronchial asthma, atopic dermatitis, type I diabetes, ulcerative colitis and Crohn disease (Klement et al., 2004, Schack-Nielsen and Michaelsen, 2007).

Another one of the most consistent findings of breastfeeding is a positive effect on later intelligence tests with a few test points advantage for breastfed infants (Uauy and De Andraca, 1995; Gomez-Sanchiz et al., 2003, 2004; Schack-Nielsen and Michaelsen, 2007). A meta-analysis of several studies by Anderson et al. (1997), as quoted by Schack-Nielsen and Michaelsen (2007) found that breastfeeding conferred a benefit of 5.3 points in cognitive function between six months and 16 years of age compared with formula-feeding and a benefit of 3.2 after adjusting for relevant variables like sociodemographic, environmental, and biomedical factors. The authors noted that the longer the infant was breastfed, the better the scores in cognitive function. Two studies conducted by Gomez-Sanchiz et al. (2003, 2004) that measured the difference in mental capabilities in breastfed versus formula-fed children at ages 18 and 24 months, respectively, confirmed these findings. In both studies, the authors found that breastfeeding for more than four months had a beneficial effect on cognitive development at the ages measured. They do add that parental intelligence clearly influences cognitive development as well. To limit familial covariant factors, Schack-Nielsen and Michaelsen (2007) cite a study comparing breastfeeding and intelligence that was conducted among 2734 adolescent sibling pairs in 2005. The children who were breastfed scored 1.7 and 2.4 points higher in intelligence within and between families, respectively. Furthermore, the longer the child was breastfed, the higher he or she scored, as the study's results showed increased scores of 0.2 points per month of breastfeeding.

A possible hypothesis for the disparities observed in cognitive development in breastfed and formula-fed infants lies in the essential and nonessential long-chain fatty acid content in each

source of nutrition. In particular, docosahexaenoic acid (DHA) present in human milk has been shown to be necessary for retinal and brain development, as it can be incorporated into cell membranes in the central nervous system (Uauy and De Andraca, 1995; Schack-Nielsen and Michaelsen, 2007). Since plasma DHA concentrations of breastfed infants are higher than that of infants who are fed formula, present formulas are thought not to provide adequate alpha-linolenic acid to support DHA accumulation in tissues. A study of full-term infants fed either human milk or cow milk formula containing 12-18% linoleic and 0.5-1.0% alpha-linolenic acids showed that at four months of age, indexes of visual acuity were more mature in the breastfed infants (Uauy and De Andraca, 1995). Additionally, psychomotor indexes are found to be higher in breastfed infants whose mothers had been supplemented with DHA in order to increase DHA content of milk, when measured at 30 months, reinforcing the suggestion that the DHA concentration in human milk increases cognitive development (Schack-Nielsen and Michaelsen, 2007).

Aside from the composition of breast milk, other factors involved in breastfeeding could be responsible for the stimulated cognitive development. Physical and psychological behavior by the mother during breastfeeding could promote cognitive development as well. Some suggest that hormones triggered in the mother through breastfeeding, such as oxytocin and prolactin, or the actual bonding experience, could influence the mother to focus more on the infant, to use more affectionate touch, and to lessen the likelihood of maternal depression, all of which could foster cognitive development in the child (Schack-Nielsen and Michaelsen, 2007).

Health Benefits to Mother-

Breastfeeding does not only provide numerous benefits to the infant, but the mother also gains several benefits. The most immediate benefit is apparent within the first thirty minutes after delivery, the time that the infant is highly alert and can therefore successfully latch on to the breast (Lawrence, 1989). Breastfeeding in this interval is beneficial to the mother because it allows for a quick expulsion of the placenta, a physiological process that must occur within the first thirty minutes after birth. As explained previously (Mader, 2000), suckling by the infant stimulates the release of the hormones oxytocin and prolactin, which begin the process of breast milk production and secretion. The hormone oxytocin, though, also performs a vital function for delivery. While the smooth walls of the uterus are relatively insensitive to oxytocin during pregnancy, sensitivity increases when the fetus is fully developed. Sudden increased levels of oxytocin in the uterus stimulate the contraction of the uterine wall, triggering labor and delivery. Therefore, as the newborn infant suckles at the breast immediately following delivery, the oxytocin that is released by the neurohypophysis in response to that stimulus will also continue to contract the uterus. These increased contractions will speed up the process of the tearing of the connections between the endometrium and the placenta, resulting in the ejection of the placenta. If this placenta expulsion does not occur within the first thirty minutes of birth, a complication termed retained placenta, the mother will be in danger of excessive bleeding. Therefore, when a mother puts the newborn to her breast during the first thirty minutes after birth, a time when the infant is highly alert and receptive to touch (Lawrence, 1989), she decreases the catastrophe of a postpartum hemorrhage (Ladewig, et al., 2010).

One of the most commonly reported benefits of breastfeeding for the mother is maternal weight loss. A large prospective cohort study conducted by Baker et al. (2008) sought to answer many questions, such as how quickly a woman can return to her pre-pregnancy weight while

breastfeeding and if the amount of weight gained during pregnancy affects one's ability to lose weight while breastfeeding. The researchers collected data on pregnant Danish women during the first trimester of pregnancy, and interviewed them by phone at two prenatal (12 and 26 weeks of gestation) and at two postpartum (6 and 18 months postpartum) time points. Based on self-reported data on pre-pregnancy weight and height, the researchers categorized the ~36,000 women by body mass index (BMI) value as underweight, normal weight, overweight, obese I, and obese II and III. During each of the interviews, the women reported their weight gain or loss. At the postpartum interviews, the women also related the method of infant feeding. The authors observed that the more the women breastfed, the lower the amounts of weight retention were at both postpartum interviews. They conclude that at six months postpartum, normal-weight women who have exclusively breastfed their infants are predicted to retain 2 kg less than women who gained a similar amount during pregnancy but breastfed for less than a week. Obese women are also expected to return to pre-pregnancy weight at six months postpartum. This study is significant because of it has several strengths: a very large cohort, prospective data collection, careful collection of infant feeding data, and others. The results are also noteworthy since they show the relationship between breastfeeding, gestational weight gain, and postpartum weight retention. Similar results were also found in a study, conducted by Kac et al. (2004), of a cohort of Brazilian women. Although the studies are somewhat limited because they do not take into account the mothers' eating and exercise habits, the significant difference in weight retention in breastfeeding and non-breastfeeding women in such a large cohort still reveals an association between breastfeeding and postpartum weight loss. In a society where obesity has become an epidemic, especially among women, research indicating a method to reduce postpartum weight retention may help decrease the number of overweight women.

Perhaps the most major benefit that mothers may receive from breastfeeding is a reduced risk of developing breast cancer. In an extremely large international study analyzing data of many different smaller studies (Collaborative Group on Hormonal Factors in Breast Cancer, 2002), results indicate that the risk of breast cancer is reduced by 4.3% for women who breastfed for a cumulative total of 12 months and by 27% for women who breastfed for a cumulative total of 55 months or more. In one hospital-based case-control study in Shandong Province, China, Zheng et al. (2008) investigated the relationship between lactation and breast cancer risk. Four hundred and four women with histologically confirmed incident breast cancer cases, along with an equal number of controls, were included. The women were all interviewed by blind interviewers and were asked whether they had ever been pregnant and how many live births they had, at what ages did they give birth, and whether they had only breastfed or gave their children formula since birth. If the respondents had breastfed, they were asked to report how many months on average they had breastfed each child. After calculating results, the authors found "a significant inverse association between lactation and breast cancer risk". They found that the mean duration of lactation per child was significantly associated with reduced risk of breast cancer for women who breastfed for more than two years per child compared to women who breastfed for less time. This reduced risk is associated with a longer duration of breast-feeding for both pre- and post-menopausal women, although the risk was generally lower for pre-menopausal women. The protective effect of breastfeeding noted in post-menopausal women implies that the benefits of breastfeeding are maintained for years after women wean their youngest children. This is significant because breast cancer risk increases with increasing age (Shema et al. 2007), so breastfeeding may reduce the risk of cancer even in the period of high occurrence. A similar study conducted by Shema et al. (2007) of ~800 Israeli Jewish women

produced comparable results. This group of authors added that the decrease in breast cancer risk with longer lifetime duration of breastfeeding is not linear, as most of the protective effect of breastfeeding is gained during the first year of breastfeeding. Additional length of breastfeeding did confer some benefit in terms of decreasing breast cancer risk. Mechanisms that have been proposed to explain the observed association between breastfeeding and breast cancer risk (Zheng et al., 2000, Shema et al., 2007) are: (1) a reduced exposure of breast cells to cyclic hormones of reproductive life because of ovulatory suppression that occurs with prolonged breastfeeding, (2) direct physical changes in the breast that accompany milk production, (3) a reduction in the concentrations of toxic organochlorines in the breast, and (4) an expression of change in growth factor beta during lactation, a negative growth factor in human breast cancer cells.

However, not all results of studies on the relationship between various lactative factors, such as number of children breastfed, mean duration of lactation for each child breastfed, and lifetime duration of lactation, and breast cancer risk have been consistent. As referenced in Shema et al. (2007), some studies suggest that the inverse relationship between breastfeeding and breast cancer risk only exists among pre-menopausal women, especially with long durations of breastfeeding and early age at first breastfeeding. Others imply that breastfeeding only confers the benefit of a reduced breast cancer risk in post-menopausal women. Still others find no association at all. Nonetheless, the overwhelming majority of studies do indicate that breastfeeding does reduce the risk of breast cancer.

Whereas studies conducted in China and Japan have all shown that breastfeeding and breast cancer risk are inversely associated, it is the results from studies of western populations that have been conflicting (Zheng et al., 2000). This could be due to the fact that studies investigating breastfeeding in western populations are usually rather limited. Most women in western cultures have two or less children, and breast-feed for an average of four months per child, usually with formula supplementation. Interestingly, a study conducted of Nigerian women (Huo et al., 2008), also investigating the association between breastfeeding and breast cancer risk and finding an inverse relationship between the two, noted that younger women in their study had an increased risk of developing breast cancer. The authors hypothesized that because the majority of older Nigerian women almost always breastfed their babies, usually for 12 months or longer per child, breast cancer did not used to be so prevalent in Nigeria. However, the younger women have begun to adopt a more western lifestyle, changing their reproductive factors to have less children and breastfeed for less amount of time per child, and therefore the protective effects of breastfeeding have diminished.

Conclusion and Summary-

While this paper has shown the many salubrious effects of breastfeeding for both infants and mothers, using formula or alternative sources of nutrition is not necessarily unhealthy for infants. Most infant formulas available in the market today are nutritionally adequate, however, as this paper has elucidated, do not provide any remedial or preventive benefits as breastfeeding does (Sarkar, 2004). For example, while formula does contain necessary nutritional contents mimicking breast milk as much as possible, human milk oligosaccharides are impossible to replicate exactly because of their diversity and complexity. Consequently, infants who are exclusively fed formula will not receive the health benefits that oligosaccharides in breast milk provide. Also, infants who are not breastfed do not have the same bonding opportunities with their mothers as infants who are breastfed do, and thus will not experience the same

psychological closeness to their mothers. Finally, mothers who choose not to breastfeed are missing out on the numerous benefits for them that come from the act of breastfeeding. In conclusion, breastfeeding is considered a superior source of nutrition over other modified milk formulas because of its many intrinsic nutritional and salutary properties, and because no formula can duplicate breast milk completely. However, in the case of absence or insufficient production of breast milk, infant formula is a suitable and convenient substitute, as long as one is careful to prepare it properly, sterilizing the feeding equipment to avoid contamination.

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Pompe's Disease and the Effects ofAlpha-Glucosidase Deficiency

Aaron Richler

Overview:

The energy that the body needs in order to function is obtained from carbohydrates that we get through our diet. These carbohydrates are monosacharides, disaccharides and polysacharides. The polysaccharides and disaccharides are hydrolyzed to monosaccharide's such glucose (which comprises roughly 80%) fructose and galactose. Most cells convert the fructose and galactose to glucose. The body can use the glucose or store it. If energy is needed, glucose can be oxidized through the many reactions of glycolysis which gives a net production of 2 ATP and 2 NADH from one molecule of glucose. In the presence of oxygen the product of glycolysis pyruvic acid will be decarboxilated to Acetyl Coenzyme A (Acetyl COA) in the mitochondrial matrix forming carbon dioxide and 2 NADH in the process. The acetyl COA subsequently enters the citric acid cycle (the Krebs cycle) where after a series of reactions, 2 molecules of ATP, 4 molecules of carbon dioxide, 6 NADH and 2 FADH2 are produced. The NADH's enter the electron transport chain and over a series of reactions with the proteins in the mitochondrial matrix form 3 ATP per molecule of NADH and 2 ATP per molecule of FADH2. (Murray)

In accordance with homeostasis, the presence of high energy will cause the body to balance energy levels by forming storage molecules of energy for use in times of need. This storage form of glucose is called glycogen; the synthesis is called glycogenesis. This glycogen can later be degraded into is monomer units for the use in oxidative phosphorylation. (Champe)

Glycogen is a branched chain of alpha-D-glucose. Most of the glucose molecules are attached to each other via a carbon to oxygen to carbon bond known as a glycosidic bond. In the linear portions of glycogen the glycosidic bond is known as the 1,4 linkage this is a bond between the carbon labeled 1 and the carbon labeled 4 on the next glucose. Every 8-10 glucose molecules there is a 1, 6 glycosidic bond causing the above mentioned characteristic branching of glycogen. (Murray) [Figure 1]

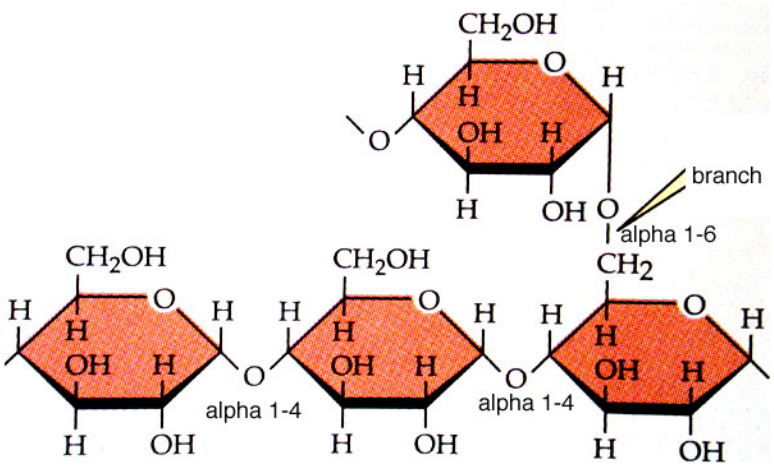

Figure 1 1,4 and 1,6 Glycosidic Bonds in Glycogen (Brooklyn College, 2009)

Glycogen is mainly stored in the skeletal muscle and the liver while it has been found in almost every cell in the body in trace amounts. The liver cell has the most glycogen consisting of up to 6% of its total weight. Alternatively in muscle cells glycogen is never more than 1% its total mass. However, because the total mass of muscle in the body is larger than that of the liver, overall, muscle contains more glycogen than the liver. (Murray)

Muscle contains glycogen for its own use. When in need of energy it will break down the glycogen via glycolysis in order to synthesize ATP for muscle contraction. Muscle will replenish

its reserves of glycogen after exercise for example and will not be affected during short periods of fasting. Yet, the liver stores glycogen in order to maintain blood glucose between meals; higher levels of glycogen are found after meals and lower levels during fasting. (Murray)

There are five enzymes that catalyze the reactions of glycogenesis. The first enzyme glucokinase which is found in liver cells and beta cells of the pancreas phosphorylates glucose. Additionally, hexokinase, a similar enzyme phosphorylates glucose in most of the other cells of the body. This phosphorylation creates Glucose 6-PO4 which is the substrate for the enzyme phosphoglucomutase which transfers the phosphate group to the 1st carbon. Next, uridine triphosphate (UTP) a high energy molecule reacts with glucose 1 PO4 and the enzyme UDP-pyrophosporylase forming UDP-glucose. Being that glucose cannot be added to other single molecules of glucose UDP glucose attaches to glycogenin a molecule with a tyrosine side chain to form the primer for additional glucoses to be attached. After adding two molecules of glucose to this base stand, the enzyme glycogen synthase continuously acts with UDP-glucose to form a long linear chain of glucose attached via alpha-1,4-glycosidic bonds. The ends of the chain to which this enzyme attaches to are called nonreducing ends. Branches are formed in the chain in order to increase solubility and to increase nonreducing ends. By increasing non reducing ends enzymes can easily remove and attach glucose molecules thereby increasing the rate of synthesis as well as degradation of the chain. (Champe)

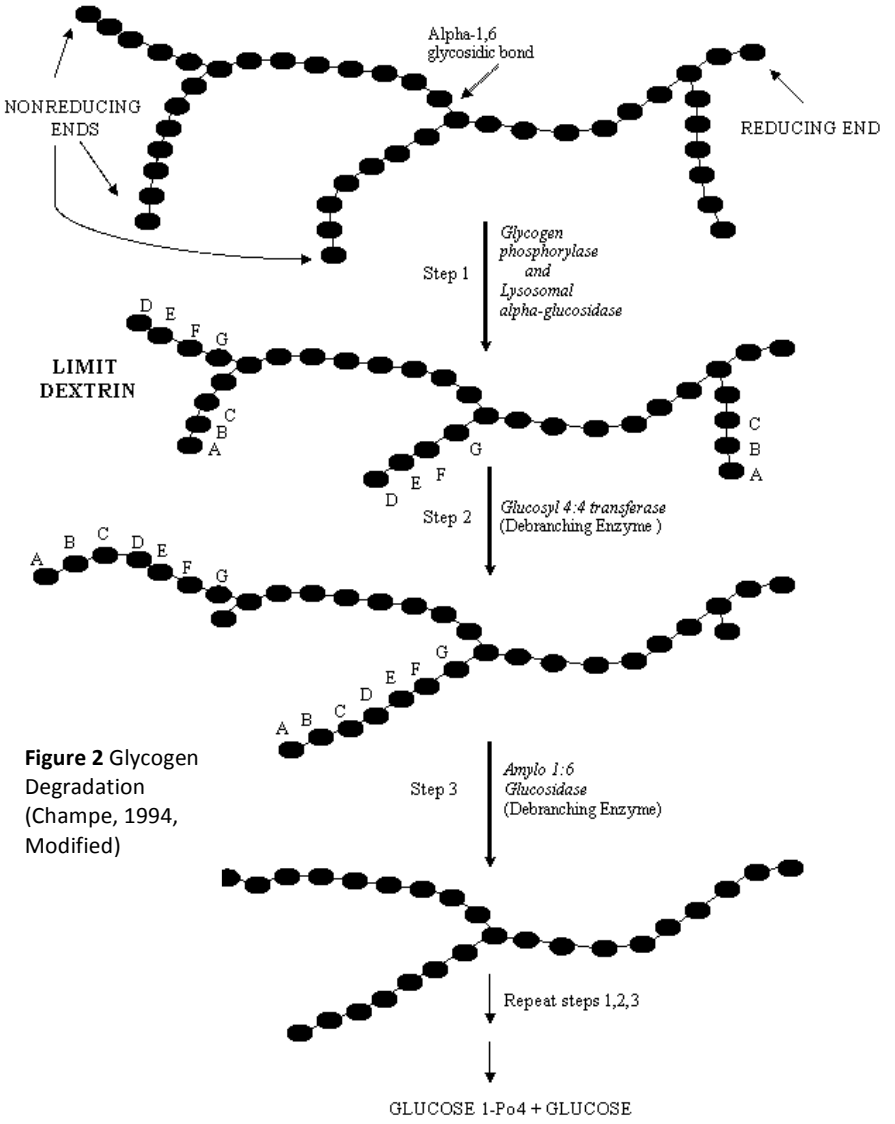

Figure 2 Glycogen Degradation (Champe, 1994, Modified)

The branches are formed by the enzyme glucosyl 4:6 transferase. The enzyme breaks a 1,4 bond in the linear portion of the chain usually forming 5-8 glycosyl chains and attaches this small chain to another spot on the chain via a 1,6 linkage. This results in two more nonreducing ends.

The new reducing ends can then be elongated by glycogen synthase and glucosyl 4:6 transferase usually removing 5-8 terminal molecules and adding them to another point in the chain by 1, 6 linkage and so on forming branched glycogen.(Champe) The remaining branched chain of glycogen after the action of glycogen phosphorylase is called the limit dextrin. Next, the enzyme glucosyl (4:4) transferase

removes 3 of the four remaining glucose units before the branch point and adds them to the remaining linear chain via a 1,4 linkage. Then the enzyme amylo-alpha-(1,6)-glucosidase cleaves the one remaining glucose molecule attached via 1,6 linkage before the branch point to which the cycle starts again with the glycogen phosphorylase removing linear portions of the chain and so on until the glycogen molecule is completely degraded. (Champe)

In response to low blood glucose glycogenolysis is initiated. First the enzyme glycogen phosphorylase cleaves the alpha-1,4 glycosidic bonds of the linear portions of the chain of glycogen. This enzyme stops when there are four glucose units left before a branching point. The enzyme Alpha-1, 4-glucosidase (lysosomal enzyme) is another enzyme that cleaves the alpha-1, 4-glycosyidc bonds until there are four glucose units left in that chain before the branch point. (Champe)

The remaining branched chain of glycogen after the action of glycogen phosphorylase is called the limit dextrin. Next, the enzyme glucosyl (4:4) transferase removes 3 of the four remaining glucose units before the branch point and adds them to the remaining linear chain via a 1,4 linkage. Then the enzyme amylo-alpha-(1,6)-glucosidase cleaves the one remaining glucose molecule attached via 1,6 linkage before the branch point to which the cycle starts again with the glycogen phosphorylase removing linear portions of the chain and so on until the glycogen molecule is completely degraded. (Champe) [Figure 2]

The products of glycogenolysis are Glucose-1-phosphate and glucose. Glucose-1-phosphate is converted to glucose -6-phosphate by phosphoglucomutase. In the liver Glucose-6 po4 is converted to glucose by glucose-6-phophotase and then released into the blood. In skeletal muscle Glucose-6-po4 is converted to glucose which is used for energy in the form of ATP from glycolysis.(Murray)

Regulation of Glycogen synthesis and degradation in the body occurs through allosteric regulation. This is charachterized by the inhibition of enzyme pathways by changing the shape of a molecule so that it cannot work or activation of a protein to make it work.

Glycogenesis will be initiated when an increased concentration of glucose 6-PO4 is in the cytosol of the cell. The enzyme Glycogen synthase is allosterically activated via positive feedback. This causes the enzyme to synthesize glycogen via pathway mentioned above.

With increased concentration of glucose, glucose 6-PO4, and ATP the enzyme glycogen phosphorylase is inhibited via negative feedback which stops the degradation of glycogen. (Champe)

Glycogen degradation

In order to break down glycogen in a particular cell the glycogen synthesis pathway must be inhibited. This inhibition is carried out by cAMP pathway/ Adenylate cyclase pathway.

In response to hormone such as glucagon attaching to it the membrane receptor couples with the G-protein in the membrane and activates adenylate cyclase enzyme in the membrane. When activated the enzyme catalyzes the reaction of ATP to form 3,5, Adenosine monophosphate (cAMP). CAMP then activates a protein kinase which then phosphorylates a

particular protein substrate in the particular pathway that is occurring. This phosphorylated protein can then act on other substrates. (Champe)

Another process that uses the cAMP pathway is the inhibition of glycogen synthesis. In response to glucagon, epinephrine, or low levels of energy glycogen synthesis will to be stopped. Glucagon or epinephrine activates adenylate cyclase and eventually the cAMP activates a kinase that inactivates glycogen synthase by phosphorylating it. This inactivated enzyme is now referred to as Glycogen synthase b. In the phosphorylated form the glycogen synthase b cannot synthesize more glycogen. (Champe)

Activation of Glycogen degradation is also carried out by cAMP pathway by the binding of glucagon or epinephrine. Activated cAMP dependent kinase phosphorylates and activates phosphorylase kinase. Phosphorylase kinase then phosphorylates the enzyme *glycogen phosphorylase b* to *glycogen phosphorylase a* which starts hydrolyzing glycogen. (Champe)

GSD

The first known diagnosis of GSD occurred in 1928 by the physicians Snappes and Van Crefald. The case involved was a 7 year old presenting with hepatomegaly or liver tumor and the patient had a very low fasting glucose yet very high acetone and beta-hydroxybuterate in the urine. After various tests the term "insufficient mobilization of glycogen" was used and the disease was defined as a debranching enzyme deficiency. Later, this disease was termed GSD III or Cori's disease. Over the next few years more cases of GSD were found by various physicians and chemists that found increased amounts of glycogen in patients' organs. (Fernandes)

There are more than 14 known glycogen storage diseases. All of these diseases are genetic and characterized by an abnormal type or quantity of glycogen in tissues, usually caused by a missing or defunct enzyme in the pathway. Nearly all of the proteins involved in glycogenolysis and glycogenesis have been the cause of a particular GSD. The defunct or missing enzyme can affect the liver function or muscle function or both. The diseases are diagnosed acc. to types of symptoms that are evident in the patient. (Chen).

Glycogen storage diseases have three general groups hepatic, myopathic and miscellaneous. Clinically, the diseases are numbered but only the first through seventh are traditionally used; the others are described according to enzyme affected (Harrsisons).

Hepatic types are diseases that are grouped acc. to their hepatic hypoglycemic pathophysiology in that those diseases that affect the liver affect sugar levels too because the liver is mainly responsible for carbohydrate metabolism (Chen). These are Von Gierke's disease (Type I) a glucose-6-phosphotase deficiency affects liver, kidney and intestine because of increased glycogen storage which causes severe hypoglycemia, fatty liver and hyperlacticacidemia, Cori's Disease (Type III) which is a debranching enzyme deficiency causing accumulation of branched polysaccharide with symptoms such as hepatomegaly, hypoglycemia and hypotonia and Hers' disease (Type VI) which also has high liver glycogen content and hypoglycemia. Others include Andersen's disease (Type IV) which is caused by a lack of branching enzyme as well as Type IX which is caused by a phosphorylase kinase deficiency. These diseases have characteristic symptoms of expected liver and sugar diseases such as hypoglycemia, hepatomegaly (Chen)

The myopathic type is a group of muscle-energy pathophysiology related diseases. These diseases are McArdle's disease (Type V) a skeletal` muscle glycogen phosphorylase deficiency with symptoms such as myglobinuria, muscle pain, muscle cramps and muscle enzymes in serum after exercise., Tarui's disease (Type VII) which is to type V but also can have symptom of hemolytic anemia. The miscellaneous group includes of Anderson's (Type IV) a branching enzyme deficiency and Pompe's (Type XI). These are different in that their symptoms and causes are not specifically related to any group (Chen). There are other GSD's that are characterized by deficiencies of cAMP dependent protein kinase or adenlyl cyclase.

The overall frequency of glycogen storage disease in populations of European decent is around 1 in 23,000 births with types I,II,III,VI, and IX the most common comprising of approximately 90% of all cases. Inheritance patterns of disease are generally autosomal recessive. However, there are forms that have been found to show x-linked patterns. (Chen)

GSD Type 2 – Pompe's

Glycogen storage disease type ll was discovered by Pompe in 1932 when a 7 year old girl was found with massive accumulation of glycogen in her tissues. The discovery made Pompe the third physician to discover a GSD. In that same year and in the years to come many more cases of cardiomegaly, hypotonia and death before the age of 1 were found with physicians reporting additional symptoms associated. (Hirschorn)

The disease is known as Glycogen storage disease type 2, Pompe's Disease, acid maltase deficiency, acid α-glucosidase deficiency or lysosomal enzyme deficiency. Initially the disease was only referred to as idio cardiomegaly but in 1963 with new biochemical discoveries in cell biology and metabolic pathways it was termed as a glycogen storage disease (Chen). Additionally, it was termed a lysosomal storage disease when Hers eventually defined a lysosomal storage disease as having 1) lysosomal enzyme deficiency 2) deposit build up present in vacuoles (Chen). The discovery by Pompe also led to the startup of research into lysosomal storage diseases with over 40 known today.

The lysosome was first discovered by Christian de Duve in 1955 as a membrane bound vacuole in human cells containing hydrolytic enzymes all active at acid pH (de Duve). These enzymes he postulated were able to break down macromolecules one of which is glycogen. (Campell)

Pompe's disease is caused by a genetic deficiency of the enzyme alpha-1,4-glucosidase (lysosomal enzyme) which is found in the lysosome and primary and secondary vacuoles of cells. Normally this enzyme as well as glycogen phosphorylase cleaves the alpha 1,4 glycosidic bonds of the branched chains of glycogen until there are 4 glucose molecules left before the branching point where the enzyme glycosyl 4,4 transferase cleaves the glucose molecules until the branch point. But a deficiency in the lysosomal enzyme will cause a build up in the glycogen branches because of the inability of glycosyl 4,4 transferase to function on the glycogen. This leads to a build up of partially branched glycogen in the cell cytoplasm as well as glycogen accumulation in the primary and secondary vacuoles of the cells. (Chen)

A postulated mechanism of action that causes the symptoms of the disease is that the deficiency of alpha-glucosidase causes a build up in the lysosomes of the cell. After reaching a capacity as well as with mechanical action of muscle the lysosomes rupture spilling their contents into the cytosol. (Griffin) An increase in protein breakdown has been seen in various studies by both radioactive isotope and stable tracers. This breakdown occurs because of the cells' low levels of energy which initiates a cellular response by harvesting energy from the muscle protein and thus the wasting away of muscle and the subsequent symptoms such as muscle pain and hypotonia as well as respiratory problems are seen. (Bodamer)

The clinical representation of Pompe's shows a broad range of phenotypes all of them include myopathy but differ in other symptoms. There are three general groups of GDS 2 infantile onset, juvenile onset and adult onset. The groups differ in their progression characteristics but all are characteristically confirmed by the absence or lack of the alpha-glucosidase. Yet, studies have shown that severities of disease are in correlation with the lack of alpha-glucosidase, with infants and children usually having the worst forms. Additionally, symptoms seem to develop when there is less than 30% enzyme activity and increase in severity with the lessoning of activity. (van der Ploeg)

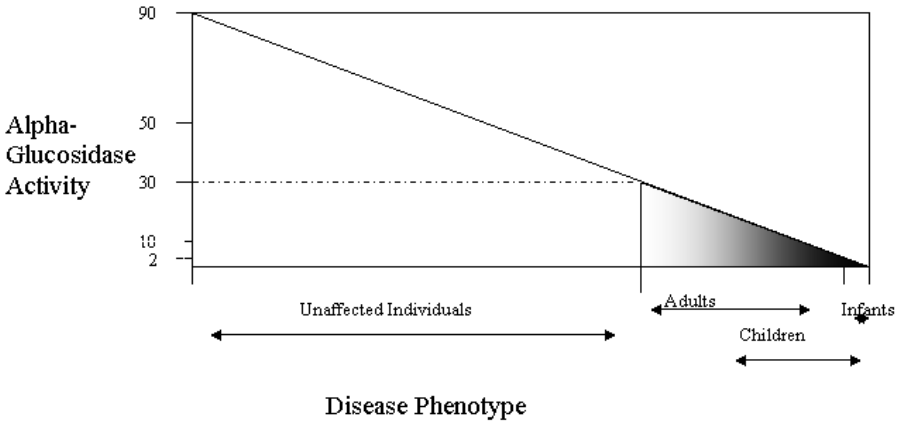

Figure 3 Model Depicting that signs of Pompe's emerge when enzyme activity is below 30% (van der Ploeg, 2008, modified)

Infantile Onset:

Infantile onset, otherwise known as the classic Pompe disease is the most common and the most severe of the three types of GDS2, causing cardio-respiratory death before the age of two years old. Most frequently it is reported in infants in the first months of life. Symptoms are generally severe cardiomegaly and hypotonia as well as macroglossia and rapid progressive weakness. Upon EKG testing as well as during autopsy it was found that both of the ventricles of the heart have increased thickness caused by the excess glycogen. (Chen) [Figure 4]

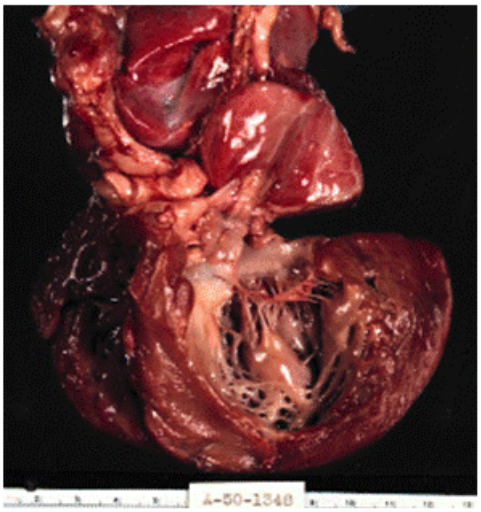

Figure 4 Severe Cardiomegaly in 9 month old child (Johnson, 2009).

Childhood-Juvenile Onset:

The childhood juvenile onset group is characterized by symptoms occurring after the age of two years old (Chen).

The symptoms usually present are skeletal muscle involved without any major cardiomegaly. The disease progresses more slowly than that of the first type and patients usually die of respiratory failure before thirty years of age. (Chen)

Adult Onset:

Adult onset GSD2 presents as a slowly progressive disease. The disease is usually found in patients in their mid 20's to 60's. In all cases known so far the disease does not cause cardiomegaly but affects other skeletal muscles as well as the diaphragm. Weakness is usually first followed by lower back pain and loss of deep tendon reflexes. Other symptoms include somnolence, orthopnea, exertional dyspnea, and morning headache. (Chen) Death usually occurs from respiratory failure but being that the disease affects the respiratory muscles death may be caused by pulmonary hypertension and cardiac failure.

Documented cases of patients that were athletically active, shows that patients can have completely normal lives before the onset of this disease.

GSD 2b: Danon's disease

First discovered in 1981 in two boys by Morris J. Dannon, this subtype is characterized by patients with GSD2 symptoms such as cardiomyopathy with vacuolar storage of glycogen yet, interestingly, normal alpha-glucosidase activity. Other clinical symptoms are arrhythmias, mental retardation and a Creatine kinase rise to tenfold in some cases. Yet, the hallmark finding is that the vacuoles contained autophagy material with glycogen in skeletal and cardiac muscle. The disease has been shown to be caused by a deficiency of a major lysosomal membrane glycoprotein called lysosomal-associated membrane protein 2 (LAMP-2) with studies mapping the deficiency as originating from the X chromosome and showing x-linked heritability patterns. (Piotrowska-Kownacka)

Genetic causes

The gene that codes for the enzyme alpha-glucosidase is located on chromosome 17 (q21-23) and is designated GAA on the human gene map and has 20 exons, 19 introns and is approximately 20 kb long. There are over 180 mutations that exist with that number increasing. The mutations that are occurring are missense, nonsense, splice site mutations, and small deletions and insertions. However, around half of all the mutations found are due to missense mutations. (Hirschorn)

In 1990 the gene was first cloned and cDNA strands were isolated. It was found that the enzyme created from the cDNA showed the same characteristics as the endogenous enzyme. (Hoefsloot)

Interestingly, carriers of the disease show codominance in that they will have 50% functioning enzyme. Therefore they will not have symptoms of the disease as mentioned above that patients with more than 30% functioning enzyme are not affected. (van der Pleug)

The three clinical manifestations of the disease prompted investigators to conclude that the various phenotypes of the disease are caused by different locations of the various mutations. For instance, in 75% of the patients that had the slow progressive type of Pompe's (which would include child and adult onset) were found to have the particular chromosomal mutation c.-32-13T>G. Yet the other more severe forms were found to have other mutations such as c.1935C>A and c.2560C>T which are found in patients with no enzyme activity at all. (van der Pleug) Additionally, researchers found that the symptoms of the other 25% of the patients with the c.-32-13T>G mutation showed varying amounts of functional enzyme leading to the possible theory that there are modulating factors involved such as diet and exercise. (van der Pleug)

Testing:

Being that in all cases of Pompe's disease there is a deficiency of enzyme prenatal diagnosis is possible in groups that are at risk. Testing of the chorionic villi cells either cultured or uncultured during pregnancy with the artificial substrate 4-methlyumbelliferyl-alpha-D-glucopyranoside (4MUG) will give a probable diagnosis for infantile onset GSD2. This testing is advantageous because it allows for the diagnosis with only 12 weeks into pregnancy and one day to actually diagnose. (Chen) Additionally, a study in Thailand successfully tested an infant with electron microscopy of chorionic villi. Also, amniocenteses as well cordocenteses is possible. (Phupong)

The artificial substrate 4MUG is also used in testing cultured skin fibroblasts. Skin fibroblasts contain large amounts of enzyme so if there is a deficiency it will be noted in accordance with strict standards. This test is advantageous in order to be able to use the same specimen over long periods of time. (van der Plaug)

This same artificial substrate can be used in muscle biopsies. Muscle biopsies can differentiate between the infantile onset and adult onset.

Additional testing includes the periodic acid Schiff (PAS) which tests for glycogen. The mechanism occurs through the reaction of periodic acid and glycogen which gives aldehyde products which then react with the Schiff reagent which gives off a purple color. (Kiernan)

Treatments:

The first study on the treatment of Pompe's was done in a Japanese lab. They subsequently showed that they can restore some activity of the acid alpha glucosidase in a mouse model (Kikuchi). Five years later in 2003 the company Genzyme created cloned enzyme recombinant human acid alpha glucosidase (rhGAA) from transgenic rabbit milk or CHO cells and have shown great results. When either of these was given in high doses of 10mg/kg there was a significant diminishing of glycogen in the cells of skeletal and smooth muscle as well as heart and other organs. These results made it possible to apply for FDA testing. (Koeberl)

Studies show that there is a critical threshold of about 30% of the average enzyme activity needed to prevent the symptoms of the disease. Enzyme therapy and replacement are in order to bring the patient up to the threshold but it seems that diet and exercise might lower this threshold. (van der Ploeg)

In 1983 Slonim and coworkers used a high protein diet to cure and raise the muscle function of a child with GSD 2. (Bodamer) This diet relied on the fact that the cell was utilizing energy from the breakdown of protein which is discussed above. Since then however, there is dispute whether such a diet would work, with recent reports showing that only 25% of patients show slight improvement of muscle function. Yet, even if this diet would work it is hard to get patients to eat a 30% protein diet. Furthurmore even if the patients can handle such a diet the patients gain considerable amount of wait which decreases their respiratory function thus removing any positive reasons for the diet.

A recent study was done on the efficacy of rhGAA type called Myzozyme® in the treatment of infantile onset. The study showed that the younger the patients were when the therapy started the better their conditions would be. Infants younger than 6 months showed the best results with all of the patients surviving past 18 months and 83% ventilator free. On the other hand the group of infants that was 6-36 months old when the treatment started did not fair as well. But both groups did show thinning of the cardiac ventricular index, changes in growth and motor development. This study also established that doses 20 mg/kg cause less adverse events than 40 mg/kg of the enzyme. (Koeberl)

There are problems with the treatment of the enzyme just like other synthetic protein therapies. The main problem that impeded the approval of Myzozyme® was the fact that when patients with no GAA activity were treat4ed with Myzozyme® they developed anti-rhGAA antibodies with fatal consequences.

Yet a recent study showed the possibility of elimination of the antibodies with strict immune-modulation therapy and had successful results. Although there are various other reactions to this drug this therapy greatly increases the chance of survival of many of the patients even at later stages. (Mendelsohn) This drug is now being prescribed to Pompe's disease patients of all ages with approval from the European commission and is in the final stages of FDA approval.

There has been promising research regarding the neural deficits in Pompe's patients that was documented just weeks ago. Researchers found that in comparison to a control group mice without the GAA gene have significant glycogen accumulation in the cervical spinal cord and other parts of the CNS. The study also included phrenic nerve monitoring which showed that mice without the GAA gene had deficient neural output. (DeRuisseau) This led to the conclusion that medications should be made to affect the nervous system as well as the musculatory system.

Conclusion

GSD 2 is an inherited autosomal recessive disorder that causes various mutations on the gene that codes for the enzyme acid-alpha glucosidase. The mutation will cause a decrease or stop in enzyme activity leading to a buildup of glycogen in the lysosomes of the cells. Due to the accumulation of the glycogen, severe complications such as cardiomegaly, hepatomegaly, hypotonia and others are seen. However, there is much hope for patients with this disease with the recent approval of the medication Myzozyme® as well as the continuing research into other antidotes that is currently underway.

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Role of genetics in prediction of coronary artery disease.

Andrey Yuabov

Introduction:

Coronary arteries disease (CAD) is a leading cause of death in United States and rest of the world. It mostly involves atherogenic formation within the walls of the coronary arteries, which in turn restricts the adequate perfusion to the heart muscle. This leads to myocardial infarction and sudden death. In the past few decades the theories of coronary arteries disease pathogenesis have changed. The facts reveal that the onset of the disease can develop as early as childhood. The degree of the disease gradually progresses in stages and it is regarded as a complex, ongoing inflammatory process that begins with initial endothelial dysfunction. There are multiple risk factors that directly and indirectly impacts the development and progression of CAD such as hypercholesterolemia, smoking, hypertension, diabetes, stress , high fat meals, lack of exercise, alcohol abuse, obesity and recently chronic infections were added to the list as well. Moreover, we realize now that coronary artery disease runs in the family and there are some risk factors that can not be altered or controlled such as age, sex and heredity. We know that the first-degree relatives of people who suffers from coronary artery disease at the early age are at much greater risk that others. Coronary artery disease is considered to be highly complex heterogeneous disease that results from blended effect of multiple genes. In recent years, researchers have identified the involvement of about 400 genes that might contribute to or to protect against development of disease.(Dzau and Liew 2007). Although, the definite conformation that even half of those is actually involved is still a long way from determination. The bigger question arises from all this information. Can we take an individual genomic profile and translate it into something that's clinically useful? Rather, than discuss all 400 genes that have been implicated, this paper will focus on several of the best-understood genes in order to find their practical application. Among them are disease-causing genes, susceptibility genes and disease-linked genes. It is important to keep in mind that this situation is rapidly changing and hopefully in future genetic approaches will shape the practice of medicine in fundamental ways.

Normal structure and physiology

The heart functions as a pump that delivers blood to the rest of the body. It consists of the three main layers: endocardium, myocardium and epicardium which surrounds by pericardium. Just like all other organs it has its own blood supply which delivered via two coronary arteries that branches of the aorta. The right coronary artery (RCA) lies in the coronary sulcus and supplies blood to the right ventricle, right atrium, and some portion of posterior wall of left ventricle as well as sinoatrial (SA) and atrioventricular (AV) node. The left coronary artery (LCA) immediately branches into the left anterior descending artery (LAD) and the circumflex artery (Cx). The LAD primarily supplies blood to the wall of the left ventricle and ventricular septum as well as to the anterior wall of the right ventricle. The Cx encircles the heart and lies in the anterior interventricular sulcus and supplies blood to left atrium, lateral wall of the left ventricle and partially supplies SA node and posterior wall of the left ventricle **(Fig 1)**. Delivery of blood to myocardium is complicated by compression of intramyocardial vessels during systole, which results in the retrograde blood flow in coronary arteries during diastole. Therefore, subendocardial layer of the myocardium is more susceptible to hypoperfusion because ventricular diastolic pressure opposes the driving pressure for flow. Development of CAD will further complicate myocardial perfusion.

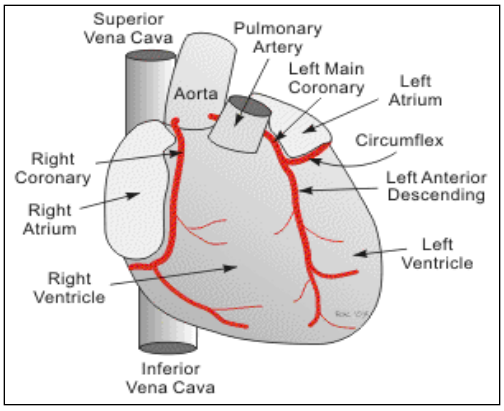

Figure 1.coronary circularion

www.cvphysiology.com/Blood%20Flow/BF001.htm

Coronary arteries consist of three layers: the innermost tunica intima, tunica media, and the outermost tunica adventitia. The tunica intima forms an inner lining of a artery and is in direct contact with blood. It is comprised of single layer of squamous epithelial cells, called endothelium that is embedded in an extracellular matrix. The internal elastic lamina separates the tunica intima from the tunica media (Fig 2).

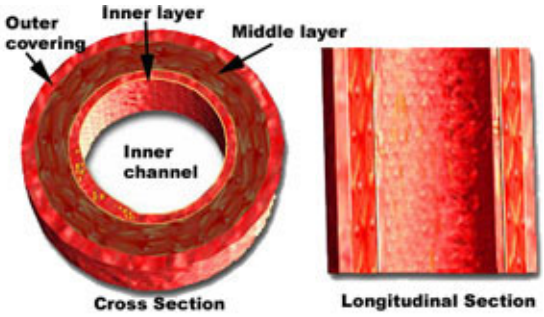

Figure 1. cross-sectional view of coronary artery
www.chelationtherapyonline.com/articles/p169.htm

Endothelial cells secret locally acting chemical mediators that influence the contractile state of the overlying smooth muscle that play a major role in the control of the vascular tone. These molecules include vasodilators such as prostacyclin, endothelial-derived relaxing and hyperpolarizing factors (EDRF), (EDHF). It also produces vasoconstrictor substances, such as endothelin and prostanoids. In addition, endothelial cells synthesize and secrete heparans and growth factor that regulates smooth muscle cell proliferation. Endothelium remains healthy by secreting different substances that prevent it from platelets aggregation or adhesion of other molecules. The media is relatively thick layer comprised mainly of smooth muscle cells and

elastic fibers. It's primary role to control the diameter of the lumen by smooth muscle cell contraction or relaxation. Tunica externa is the most outer layer and consists of elastic and collagen fibers. It contains numerous nerve ending and tiny vessels that supply tissue of the cell wall itself as well as connects the artery to the surrounding tissue. This layer separates from media by network of elastic fibers, called external elastic lamina.

Pathophysiology of CAD Atherosclerosis is the major cause of CAD. Theories of the pathophysiology of atherosclerosis underwent big changes over time. Few decades ago it was looked as mostly disorder of lipid storage, and later it was considered to be disorder of lipid metabolism. Further, CAD was looked as disease of the smooth muscle proliferation. With the discovery of inflammatory cells and molecule in plaques led to conclusion that disease might be inflammatory in nature. Current views on pathogenesis of atherosclerosis are as follows. The development of an atherosclerotic plaque is a multi step process. The presence of risk factors for CAD, such as hypercholesterolemia, cigarette smoking, hypertention, diabetes mellitus and shear stress are associated with endothelial dysfunction. These conditions gradually damage healthy and smooth lining of endothelium. This is the first step in development of atherosclerosis and it results in increased permeability of endothelium **(fig 3)**.

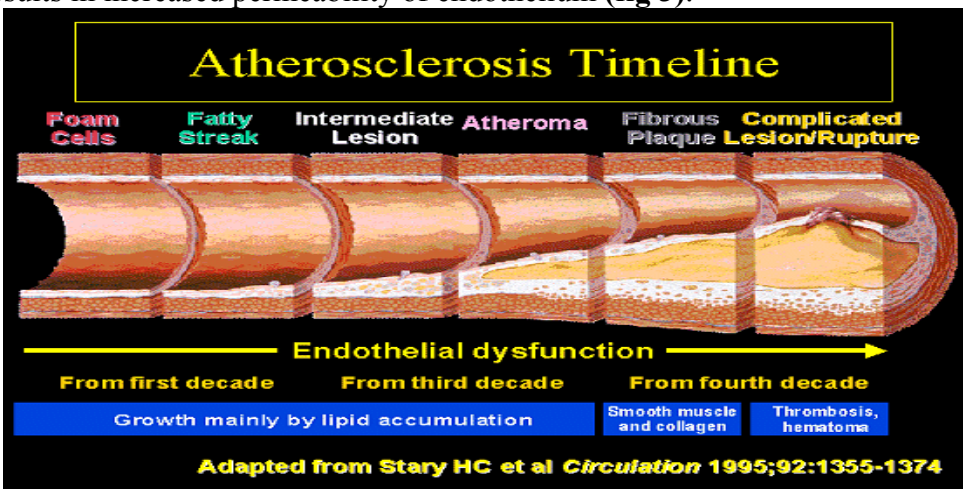

Figure (3) Atherogenesis

www.vhn.ca/images/atherosclerosistimeframe.gif

Once the layer is damaged, the excess low density lipoproteins and other cellular waste product can easily get into the inner layer of an artery wall. While entrapped low density lipoprotein is progressively accumulates and undergo oxidation. This initiates inflammatory response and stimulates smooth muscle cells proliferation. In response to invasion, endothelium and smooth muscle cells release mediators to initiate recruitment of leukocytes, T-cells and monocytes into the developing lesion. Furthermore, intralesional monocytes become macrophages. They eat and digest large amounts of surrounding oxidized low density lipoprotein. As of the result of this process, these lipid filled macrophages are becoming foam cells, due to their foamy appearance. Together with T cells and monocytes, they form the bulk of "fatty streaks". ". The next stage in development of atherosclerosis is a formation of "intermediate or fibrofatty lesion" which is more complex in composition. The combination of fatty streaks growth and ongoing inflammation, stimulate smooth muscle cells to multiply and migrate to an injury site. As these cells become noncontractile and fibrous they migrate to the top of the atherosclerotic plaque, forming a cap over it. These lesions might not be immediately harmful and may be viewed as protective response to injury. As this plaque continues to grow, the coronary arteries gets thicker,

harder and looses its elasticity. Calcium ions binding to the site of the plaque formation might contribute to lack of elasticity as well. As plaque expands into the lumen of a coronary artery, blood flow is greatly reduced through that artery. At this stage physical symptoms such as chest pains and shortness of breath may occur which associates with angina pectoris. However, it is the physical disruption of the plaque exhibits an acute coronary event such as myocardial infarction. The rupture of the plaque usually occurs due to increased pressure in a narrow artery. It might cause the complete occlusion due to blood clot formation cascade and manifest MI or sudden death.

Genomic approach to CAD

The biggest challenge for scientists of a new era of genetics is to identify the genes and gene expression changes that are responsible for pathological process of CAD. Two main projects: human genome project and international hapmap project provided a crucial data for genetic analysis in a complex heterogenic disease such as atherosclerosis and coronary artery disease. To study such as complex diseases, scientists recently have been using two general categories of study designs such as association studies and linkage studies. In Linkage studies, the goal is to identify the inheritance of the disease in large families throughout few generations. It is possible if we are able to identify single nucleotide polymorphism (SNP) on the fragment of the chromosome that is inherited by family who is affected by a disease but not those who is disease free. Furthermore in this design, we concentrate on identifying the gene variation of our interest after narrowing down the section of a chromosome. This methodology is found to be helpful especially in identifying diseased genes that inherited in a Mendelian fashion. Gene disease associated study was introduced in 1996. It is designed to identify genetic variation of available genome in associations with specific trait or disease of interest. Two independent groups are usually participate in these studies. Those groups are, "cases" which are those individuals who has been diagnosed with a disease and "control" are those who are similar people within same parameters that are disease free. After carefully calculated results, if frequencies of genetic variation are greater in individuals who are affected by a disease then it said to be associated with a disease. Positive association cannot be interpreted as causation, but only statistical association for the most part. Both of these methods have their advantages and disadvantages just like any other studies.

Throughout years of genetic study, molecular biologists have identified several disease-causing genes for disorders in lipid metabolism. They are directly related to development of coronary artery disease. In order to understand dyslipidemia disorders, it's important to understand the physiology of lipid metabolism. Lipids are hydrophobic and therefore have to be transported by lipoproteins in blood. Different lipoproteins have variety of integrated and peripheral apoproteins that perform a different functions such as structural integrity, receptor binding, and enzyme activation. Lipoproteins are categorized by their density of protein content. They divide into two major groups high density lipoproteins (HDL) and non-high density lipoproteins. Non-high density lipoproteins are further subdivided into chylomicrons, very-low density lipoprotein, intermediate density lipoproteins and low-density lipoproteins. They transport lipids from sites of absorption or synthesis to sites of their utilization. High-density lipoproteins are considered as "good cholesterol". They are responsible for transport of excess lipids in circulation back to the liver.

For example Apoprotein B mostly found on non-high density lipoprotein and involves in assembly and packaging of ingested triglycerides and cholesterol into non-high density lipoproteins. Apoprotein E involves in receptor binding to hepatocytes via (LDL receptor).

Through this mechanism, liver uptakes the necessary lipid contents from non-high density lipoprotein. Apoprotein C binds to adipocytes and skeletal muscle cell via lipoprotein lipase, which acts as the receptor to facilitate lipid absorption.

Disease-causing genes have been identified for disorders in lipid metabolism such as familial hypercholesterolemia, familial defective Apolipoprotein B, Apolipoprotein A deficiency, Tangier disease, Autosomal recessive hypercholesterolemia, Sitosterolemia ect.

Familial hypercholesterolemia (LDL)receptor

Familial hypercholesterolemia is an autosomal dominant disease defined by the presence of mutation in the low-density lipoprotein receptor gene. It characterized by severely elevated plasma level of low density lipoprotein (Saint-Jore B et al., 2000) Heterozygouse familial hypercholesterolemia is the most prevalent and affect about 1 in 500 people. Affected heterozygous individual exhibit plasma cholesterol levels in the range of 300-400 mg/dl. Clinically, patients develop severe atherosclerosis involving multiple vascular territories but especially in coronary arteries. This leads to premature coronary atherosclerosis in fourth decade of life and often requiring bypass surgery early in life. Familial hypercholesterolemia patients were found to have one copy of mutated low density lipoprotein gene, which normally is located on chromosome 19p13. A variety of point, deletion and splice mutation have been described. Effect of this mutation leads to loss of its function. The mutant low density lipoprotein receptor has reduced affinity for removal of apolipoprotein B and E from the circulation. (Marian Ali,2000) Because Familial hypercholesterolemia is not only relatively common and associated with a high risk of early coronary artery disease but is treatable with LDL-C-lowering strategies, this genetic disorder meets the World Health Organization criteria for systematic screening. It has been estimated that familial hypercholesterolemia is properly diagnosed in only 15% of affected Canadians and as many as 30% of the patients do not survive their first myocardial infarction. Therefore, early detection of the disease has the potential to save life and prevent morbidities (Yuan et al.,2006)

Apolipoproteins are important components of lipoprotein particles. Current data shows that measurements of various apoliportroteins may help to predict the risks of coronary artery disease (Walldius & Jungner, 2004).Their involvement in synthesis and metabolism of lipoproteins are gradually being defined. In addition to stabilizing lipoprotein structure, some of lipoproteins act as ligands to tissue receptors, while others activate or inhibit enzymes involved in metabolic steps in circulation or tissues.

Apolipoprotein A deficiency.

Apolipoprotein A has two major forms A1 and A2. Apolipoprotein A1 is associated with high density lipoprotein cholesterol, and may be largely responsible for determining the plasma level of it. In addition apolipoprotein A1is the ligand for ATP-binding cassette protein and hence is involved in the docking procedure by which excess cholesterol in peripheral cells is externalized to high density liporprotein (Walldus & Jungner,2004). Its been reported that deficiency of apolipoprotein A1and low levels of high density liporprotein cholesterol were significantly related to an increased risk of cardiac mortality in patients with coronary artery disease(Walldus & Jungner,2004). Gene coding for apolipoprotein A1is located on the long arm of chromosome 11. Two polymorphism of apolipoprotein A1gene namely G to A substitution at -75bp of transcription start site and C to T substitution at +83 bp have been implicated in susceptibility to coronary artery disease and shown to influence plasma lipid levels (Taranjit et al.,2008) Many studies are controversial about the effect of particular gene polymorphism on pathogenesis of

atherosclerosis and coronary artery disease, but in combination with other polymorphic variants may be better predictor of disease risk in future.

Familial Defective Apolipoprotein B

Plasma lipoproteins are important determinants of atherosclerosis. Apolipoprotein B is a large amphipathic glycoprotein that exists in two forms and plays an important role in lipid metabolism. Both forms are produced by the same apolipoprotein gene: apolipoprotein b-48, which is required for chylomicron production in the small intestine and apolipoprotein; b-100, which is required for very low density lipoprotein production in the liver. In addition, apolipoprotein b-100 is the ligand for low density lipoprotein-receptor-mediated endocytosis of low density lipoprotein particles.(Whilfield et al.,2004) Mutation in apolipoprotein b-100 is characterized by increased plasma low density lipoproteins and very low density lipoprotein levels and has clinical features of hypercholesterolemia and premature coronary artery disease(Marian.Ali.2000). Familial defective apolipoprotein b-100 is a second relatively common cause of severe autosomal dominant hypercholesterolemia (Dzau. 2007). It is relatively common, with estimated frequency of 1 out of 500 in normal population and clinically not possible to distinguished from heterozygous familial hypercholesterolemia (Whilfield et al.,2004). The most common mutation is R3500Q which involves in substitution of a glutamine for arginine at the position 3500 (Whilfield et al.,2004). Other point mutation, R3500W and R3500C are rare cause of familial defective apolipoprotein b-100 (Real et al.,2003). Low density lipoproteins of affected individual will have a 90% decrease in affinity for low density lipoprotein receptor and low density lipoproteins clearance will be markedly impaired. Therefore routine screening is feasible and should be performed not only in subjects with clinical phenotypes but also in asymptomatic subjects with elevated low density lipoprotein levels.[Ali.2000] Treatment is similar to that of familial hypercholesterolemia with reliance on statin therapy, which both decrease very low density lipoprotein production and enhances clearance of very low density lipoprotein remnants[Dzau.2007]

Other mutations of Apolipoprotein b-100 may cause familial hypobetalipoprotenimia, which is characterized by hypocholesterolemia and resistance to atherosclerosis and coronary artery disease (Whitfield et al 2004).

Autosomal recessive hypercholesterolemia is caused by mutation in a putative adaptor receptor protein called ARH, that function in the internalization of low density lipoprotein receptor and cargo. This recessive disorder is characterized by severe hypercholesterolemia, xanthomatosis and premature coronary artery disease (Arca et al., 2002). Autosomal recessive hypercholesterolemia did not associated with the low density lipoprotein receptor or apoliporpotein b genes and exhibited an autosomal recessive inheritance, with the parents of the affected subjects having normal low density lipoprotein cholesterol values [Dzau.2007]. Interestingly, the function of low density lipoprotein receptor is near to normal in their cultured fibroblast but is impaired in lymphocytes, macrophages and hepatocytes. Low density lipoprotein receptor protein is present in these cells within normal limits but dispersed unevenly with most of the receptor residing on the plasma membrane, where it binds to low density lipoprotein but fails to internalize and degrade its cargo [Dzau.2007]. This specific disorder is rare and prevalent in Sardinia, Italy. Two ARH mutation were present in all 17 unrelated Sardinians families with ARH: a frameshift mutation (C432insA) in exon 4(ARH1) and nonsense mutation (C65G->A) in exon 1(ARH2) (Arca et al., 2002) . Screening of the coding

regions of ARH with similar phenotype in other random parts of the world revealed that only four out of 40 of them were positive for ARH1 mutation and all happened to be Italian.

Low circulating serum levels of high density lipoprotein cholesterol seem to be a frequent lipoprotein disorder in coronary artery disease and can be caused by either genetic or lifestyle factors. Many gene abnormalities can cause this lipid disorder.

Tangier disease is a rare disorder of lipid metabolism, characterized by extremely low levels of high density lipoprotein cholesterol. It is caused by mutation in the adenosine triphosphate-binding cassette transporter A1 (ABCA 1), also known as the cholesterol efflux regulatory protein (Kolovou et al.,2006). ABCA1 is a member of ATP-binding cassette transporter family. These integral proteins use ATP as a source of energy to transport variety of molecules including ions, vitamins, lipids, peptides proteins and number of hydrophobic compounds across intracellular and plasma membranes (Kolovou et al.,2006). Complete loss of ABCA1 function leads to severely decreased cellular cholesterol efflux and cholesterol ester accumulation in macrophages and other cells of the reticuloendothelial systems (Rust et al.,1999). Due to impaired ability to transport cholesterol out of the peripheral cells, it accumulates in body tissues. Therefore clinically, Tangier disease patients present with hepatosplenomegaly, enlarged tonsils, neurapathy and most important in our case atherosclerotic lesions. Studies suggested that carriers of defective adenosine triphosphate–binding cassette transporter A1 bear a moderately increased risk for coronary artery disease.

Sitosterolemia is a rare autosomal recessive disorder. It characterizes by markedly increased intestinal absorption of all sterols including cholesterol, shellfish and plant sterols and impaired resecretion of those sterols into bile. Affected individual have expanded body pools of cholesterol and very elevated plant-sterol levels. Affected individual will frequently develop accelerated atherosclerosis and premature coronary artery disease (Lu et al.,2001) In the studies, it has been mapped that this condition results from over 25 mutation in either ABCG5 or ABCG8 genes which located on chromosome 2 in band 2p21. These genes encode for adenosine triphosphate-binding cassette transporter protein (ABC) transporters, steroline-1 and steroline -2. Their functions are to limit intestinal absorption and stimulate excretion of noncholesterol sterols by liver into bile. Mutation of these transporters predispose to sterol accumulation and early artherosclerosis. Sitosterolemia can clinically present similar to homozygous familiar hypercholesterolemia in terms of elevated LDL-cholesterol especially in childhood around 300 mg/dl or 8 mmol/l. Therefore, it a possibility that sitosterolemia is frequently misdiagnosed with hyperlipidemia.

Although, all mentioned above lipid metabolism disorders are rare, they are high risk factors and directly related to incidence of coronary artery disease. Studies of these disorders gave researches a better understanding of lipoprotein and cholesterol metabolism. Whether hypercholesterolemia is caused by mutation in one gene or cumulative effect of several genes defects, early diagnosis is essential to start appropriate therapy. Most notable was development of Statins drugs which is a class of medications that was developed to lower plasma low density lipoprotein cholesterol levels.

MEF2A

Recently, the first non-lipid related disease causing gene was identified and proposed to be responsible for an autosomal dominant form of coronary artery disease (adCAD). However, when association is detected, it is still difficult to prove direct causality. False positive association may arise due to differences in ethnicity, age or sex [Dzau, 2007]. Several studies were performed to identify the role of human myocyte enhancer factor-2A (MEF2A) and its mutation in early coronary artery disease development .MEF2A is a protein that is coded by MEF2A gene which is located on chromosome 15q26. The seven-amino acid deletion of the human transcriptional myocyte enhancer factor-2A was reported to be a functional mutation. It disrupted nuclear localization of MEF2A, reduced MEF2A-medieted transcription activation. In addition it abolished synergistic activation by MEF2A and by transcriptional factor GATA-binding protein which involved in cell growth, with which MEF2A interact to regulate the expression of the downstream target genes, by dominant –negative mechanism (Wang et al.,2003). One study was dealing with large family with 13 patients who had an autosomal dominant patterns of CAD. Nine of 13 patients later developed acute myocardial infarction. Further genome wide association study linked data to chromosome 15q26. MEF2A also demonstrate its relevance to expression of coronary arteries endothelium (Wang et al.,2003). Another independent case-control studies was performed from 2003 to 2007 involving 726 individuals in China (Han et al.,2007). The results of that study suggested the disease-causing relationship between MEF2A and coronary artery disease. In subsequent study, Weng et al who report sequencing MEF2A in 300 patients with premature coronary artery disease and control, only 1 CAD patient was reported to have the mutation that wasn't found in the control group (Altshuler & Hirschhorn, 2005). Also another study in large Irish population showed no association between variation in the MEF2A gene and coronary artery disease (Horan et al.,2006).However, the absence of CAD in a control group wasn't confirmed by angiography. As science progresses it is becoming much easier to collect data but it also causes many controversy in its analysis. MEF2A plays an important role in cardiovascular biology, but its genetic variation contribution to pathophysiology in CAD should be further validated in order to make it the criteria for genetic testing for CAD.

Susceptability genes are those that increase or decrease the risk of developing disease and in combination with environmental factors and other genetic factors may contribute to pathogenesis of a disease. These genes might have less predictive values for developing a disease, but contribute a lot to understanding of pathophysiology process.

eNOS gene.

Nitric oxide (NO) is gas and is identified as endothelium derived relaxant factor. NO is synthesized from substrate L-arginine by endothelial cell nitric oxide synthase (eNOS) which encoded by nitric oxide synthase 3 gene (NOS3) that is located on 26-exon on chromosome 7. NO plays a few important roles in healthy endothelium such as regulation of vascular tone in coronary arteries, prevents leukocytes adhesion and platelets aggregation and inhibits vascular smooth muscle migration and proliferation. Biochemical and/or physical shear stress damages endothelium and impairs production of mediator such as nitric oxide. This might lead to vasoconstriction, loss of elasticity and promote platelets activation. Indirectly decrease in nitric oxide activity affect conditions such as hypertension and hypercholesterolemia which predispose to atherosclerosis [Dzau.2007]. Few clinical studies indicated relationship between different

eNOS gene polymorphism and atherosclerosis development. The most commonly studied variant is Glu298Asp which is conversion of glutamate to aspartate at the position 298 that results from G894T substitution within exon 7. In English study sample Glu298T variance has been reported to be associated with atherosclerotic coronary arteries. The mechanism is unknown but by altering mature protein activity can affect enzyme activity and decreased local nitric oxide synthesis. As a result of case-control study involve Iranian population (Salimi et al., 2006), we observed that the genotype frequencies differ from allele frequencies significantly between control and CAD patients. However, controversy brought up by other small studies that do not support these findings. Therefore the relevance of this data remains unclear. It might be because of subjects were picked from different ethnicity and genetic background. Further larger studies are needed in order to prove major effect of Glu298Asp polymorphism on disease development but for now it can be considered as indirect genetic marker associated with the disease.

TNF-alpha

Tumor necrosis factor alpha (TNF-alpha) is a pro-inflammatory cytokine that plays a role in pathogenesis of CAD. It was localized in atherosclerotic plaque in pathological studies. Macrophage derived TNF-alpha has important pro-inflamatory autocrine and paracrine effect. Stimulation of the cells by TNF-alpha, activate the cascade of events that lead to upregulation of various adhetion molecules. Overall contribution of TNF-alpha believed to be activation of influx of additional inflammatory cells and involvement in endothelial dysfunction (Dzau 2007). TNF-alpha has two receptors through which it exerts its effect TNFR1 and TNFR2. Polymorphisms in TNF-alpha and TNF receptor genes are hypothesized to affect secretion of this cytokine and relate to presence or severity of CAD. To investigate this matter scientist conducted a study 259 patients with angiographiclly confirmed CAD and control group (Allen et al., 2001). As a result of the statistical analysis no significant differences were found between polymorphism frequencies. Another European study was performed to investigate association of TNF-alpha gene polymorphism and its relationship with CAD via polymerase chain reaction-single-strand confirmation polymorphism and sequencing (Herrman et al.,1998). Although, TNF-alpha gene polymorphism have been associated with number of inflammatory diseases it is unlikely to contribute to development of CAD

Diseased-linked genes are those genes whose expression is linked or connected to the disease but the cause and effect relationship is not established. They might serve as biomarkers for the disease. A big chunk of genes were newly identified as disease-linked genes for CAD. The most studied include intracellular adhesion molecules-2, E-selectine gene, ect. The reason for interest in studying disease-linked genes is the fact that about half of the patients who are diagnosed with CAD do not have traditionally established risk factors. Therefore, the search for other biomarkers was proposed in order to help identify individuals who might be at risk.

Cellular adhesion molecules.

Cell adhesion molecules (CAMs) are transmembrane proteins that involve in binding of cells with other cells or extracellular matrix. In healthy endothelium, expression of adhesion molecules is very limited. In diseased coronary artery, transendothelial T-cells and monocytes migration are mediated by these molecules **(figure4) Transendothelial migration**.

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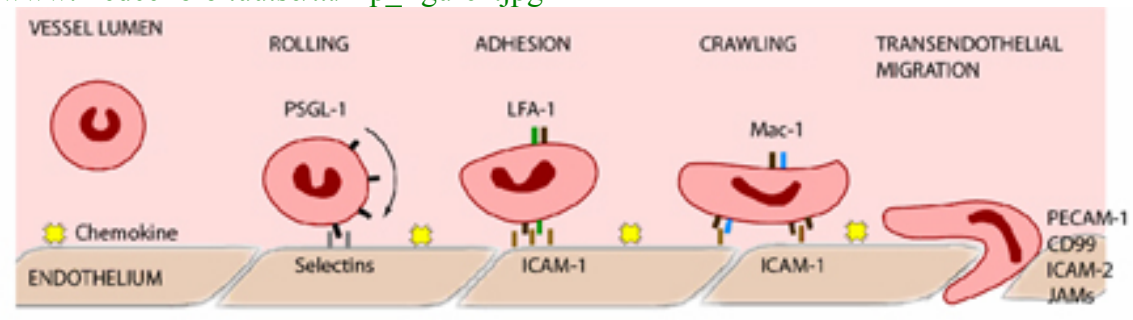

One of the CAMs category belong to Immunoglobulin superfamily. They are intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Animal and human studies earlier show that overexpression of adhesion molecules and endothelial cell activation causes accumulation of leukocytes in coronary tunica intima. According to latest reports, expressions of cellular adhesion molecules is stimulated by cytokins such as interleukin-1, tumour necrosis factor (TNF) and interferons-gamma (INF) (Ohta et al., 1999) Furthermore, the increased expression of cellular adhesion molecules in atherosclerosis shows in early stages of atherosclerotic development which presented by fatty streaks in inner layer of an artery as well as the latest fibro-fatty lesions. Linkage of levels of ICAM-1 and VCAM-1 to coronary artery disease that was established by study and can be considered as an independent risk predictor (Hajilooi et al.,2003).Although, genetic aspect of these molecules remain poorly known, recent multi-ethnic study was conducted to investigate relationship of 12/ICAM-1 and 7/VCAM-1 single nucleotide polymorphism (SNP) and coronary artery calcium and ICAM-1 SNP and circulating levels of soluble ICAM-1. It showed no significant association of ICAM-1 and VCAM-1 and coronary artery calcium in any ethnic groups, but polymorphism in ICAM-1 showed significant association with soluble ICAM-1 levels and was identified as a marker for atherosclerosis (Bielinski et al ., 2008).

Other family of cell adhesion molecules consists of E-selectin and P-selectin. Both of them suggested to play an important role in early and advance stages of coronary arthrosclerosis from earlier double-knockout mouse experiment. P-selectine is important in the transient attachment of leucocytes to endothelial cells and platelets (Carter et al., 2003). When endothelial cells get activated via mediators such as histamine and thrombin in inflammatory process, P-selectine translocates from internal to endothelial cell surface. Just like prior mentioned cell adhesion molecules, P-selectine also contributes to development of atherosclerosis by facilitating adhesion of leukocytes to injured endothelium and platelets. Soluble P-selectins were found in plasma in multiple conditions including heart disease. Number of polymorphism has been identified in gene encoding P-selectin. THR715Pro polymorphism for example was reported to be associated with myocardial infarction in previous studies. In 2003 the study was performed that compared levels of soluble P-selectin in 249 individuals with CAD and 252 healthy control group and investigated the relationship between polymorphism of P-selectin and circulated soluble P-selectin levels and cardiovascular risk (Carter et al., 2003). It showed that levels of soluble P-selectin were higher in affected individuals than control group after adjustment for age sex and smoking status. Moreover, no significant association was found between P-selectin polymorphism in CAD affected patients and healthy control group. This study suggested that the relationship between Thr715Pro polymorphism and levels of soluble P-selectins exist but its mechanism is still unclear.

E-selectin also involved in adhesion of leukocytes to activated endothelium. Mutation of E-selectin gene, due to G to T mutation (G98T) in untranslated region of exon 2, was found to be significantly higher in frequency in patients with CAD than control group and thus suggests association with premature coronary artery disease (Zheng et al.,2001).Even after adjustment for other multifactor, mutation of G98T may be an independent risk factor for premature CAD. Another study was performed in 2006 within Saudi Arab population. It attempted to investigate amino acid change from serine to arginine at codon 128 (S128R), which corresponds toA>C nucleotide change at the position 561 (A561C), in the epidermal growth factor-like domain of the E-selectin gene that was previously implicated in pathogenesis of CAD in few ethnic groups (Abu-Amero et al., 2006). The frequency of mutation in 128r allele was found to be much higher in patients with CAD than control group, but lost association with adjustment for other CAD risk factors. The most recent study that investigated Association of E-selectin gene (S128R) polymorphism in patients with CAD within Indian population was consisted with previous findings. It revealed the increased frequency of E-selectin polymorphism in CAD patients but its interaction with other risk factors showed that in fact the significant determinant of coronary artery disease were presents of diabetes, hypertension, smoking habit, elevated serum triglycerides and low HDL level.

The involvement of cell adhesion molecules was established to play an important role in development of atherosclerosis. Overall studies suggest that neither the measurements of soluble adhesion molecules, nor screening for their gene polymorphisms cannot be an independent predictor of CAD. It may only offer some additional marker of atherosclerosis but cannot go beyond the assessment of conventional coronary artery disease risk factors.

Conclusion:

Throughout last decade significant advances in genetic studies were made which gave us a fair insight on pathogenesis of coronary artery disease. It no longer viewed as a simply degenerative disease or gradually accumulating lipid disease. Current views regard CAD as complex disease that involved in active process of alteration in cell intercommunication and cell signaling. At this point in time we can only speculate on assumed biological role of genetic polymorphisms that has been identified in relationship with CAD. Field in genetic studies is promising and expects to accelerate as future studies will focus on identifying new genetic polymorphism in connection to CAD. However, at this time coronary artery disease can't be simply diagnosed through genetic testing, but can only look for variants that increase the risk of developing it. Prediction of CAD via genetic polymorphism may eventually lead to clinical utilization, but now only physician can diagnose the disease based on a medical history, family history, risk factors and the results of medical tests. Genetic testing can not clearly tell if disease is going to develop or not, but if certain genetic markers are present, it may help us to modify our estimated risk of developing it. When considered genetics along with other individual's information including other risk factors may help individual to reduce the risks that we can control such as lifestyle, diet and exercise. Moreover, if clinician knows about increased genetic predispositions, they may stronger recommend lifestyle changes. In addition, high index of suspicion might result in early diagnostic application and close monitoring of patient with marked genetic polymorphism by their doctors. Currently a few of screening tests are available for asymptomatic individuals who are at risk for premature atherosclerosis and CAD such as computed tomography, ultrasound ect.

Hopefully in near future genetic studies will lead us to revolutionary changes in further understanding, ability to diagnose, prevent and treat the wold's biggest killer.

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Vitamin D3

Aryeh Grossman

Introduction

Vitamin D is the only vitamin that is free. It can be easily obtained from exposure to sunlight and yet more than 60% of Americans are Vitamin D deficient (Ginde et al., 2009; *Bones, Vitamin D, and Calcium*, n.d). The significance of Vitamin D can't be emphasized enough and is often overlooked. Some its profound effects are apparent in the prevention of various diseases such as cancer, multiple sclerosis, and cardiovascular disease et cetera. Many lives can be spared each year just by taking Vitamin D. From cancer alone, 23,000 deaths can be attributed each year to a lack of Vitamin D (Grant, 2002; Reichrath, 2008). Vitamin D deficiency in America is an epidemic that needs to be addressed.

Vitamins:

Vitamins are required for the well being of the human body, in essence in achieving homeostasis. Vitamins are organic substances (coenzymes) that can't be synthesized by the human body. Vitamins are divided into two discrete categories: water-soluble and lipid-soluble vitamins. Water soluble vitamins can be eliminated easily from the body in the form of urine and sweat. On the other hand, an excess intake of lipid soluble vitamins can be fatal. They can't be easily excreted as a waste, due to their hydrophobic properties. Vitamin D is one such lipid soluble vitamin that is stored in adipose tissue.

Vitamin D_3 Background

Vitamin D is a steroid hormone despite it being classified as a vitamin. The molecular structure of Vitamin D closely resembles typical steroid hormones, which have the same cyclopentanoper-hydrophenanthrene root carbon ring structure. This suggests that Vitamin D reacts similarly to other steroid hormones. There are various forms of Vitamin D, but the naturally occurring form is cholecalciferol, also known as Vitamin D_3. The primarily and most abundant source of Vitamin D is obtained during the presence of sunlight. It can also be naturally obtained from several food sources, however, in minute amounts.

BiochemistryA cholesterol molecule, 7-Dehydrocholesterol functions as a precursor to Vitamin D. The highest concentrations are found in the epidermis, particularly the stratum basale and stratum spinosum layers. In conjunction with sunlight, in the form of Ultra-violet B radiation, cholecalciferol is synthesized. The most effective wavelength is found between the range of 270-290 nm. This absorption excites the double bonds, allowing the B-ring to open, thereby making it into a more flexible structure. Once produced, cholecalciferol (D_3) the inactive form of Vitamin D_3 is then transported to the liver by the plasma transport protein, Vitamin D–binding protein (DBP) or also known as transcalciferin (TC). In the endoplasmic reticulum of hepatocytes, cholecalciferol is hydroxylated at the Carbon-25 position to yield 25-

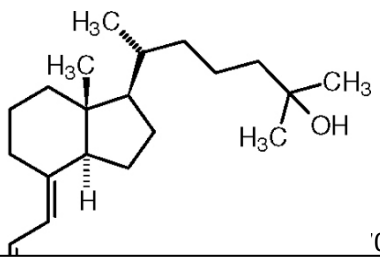

Figure 1. 1α,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$] also known as Vitamin D_3. Source: IUPAC. (Dixon, 1982)

hydroxycholecalciferol (Calcidiol) by the enzyme 25-hydroxylase. It is then successively transported by DBP to the proximal tubules of the kidney where it is catalyzed by the enzyme 1α-hydroxylase. The hydroxyl is placed on Carbon 1 or 24 to yield the following, 1α,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$] and 24*R*,25-dihydroxyvitamin D_3 [24,25$(OH)_2D_3$] respectively (Dixon, 1982). Finally, by a means of diffusion (Hollis et al., 2008), DBP transports both steroids to their target tissues, where 1α,25-dihydroxycholecalciferol or also known as calcitriol, is considered to be the hormonally active form of Vitamin D_3 (Figure 1). The inactive form 24, 25$(OH)_2D_3$, is chemically degraded and eventually excreted as either calcitroic acid or as 23-carboxyl derivatives. In comparison to its inactive isomer 24,25$(OH)_2D_3$, the affinity of calcitriol to Vitamin D_3 receptor sites is over 1000 times more than to its isomer. (Norman, 1990; 1998; MacDonald & Sutton, 2003)

Vitamin D_3 similarly to other steroid hormones, binds to intracellular ligand-dependent transcription receptors. Being hydrophobic it is easily able to penetrate the phospholipid membrane of target cells. Once in the cytosol of the cell, Vitamin D_3 binds to a receptor protein known as the Vitamin D receptor (VDR), which prompts a cascade of macromolecular interactions leading to the transcription of certain genes. Promoted by Vitamin D_3 the Vitamin D receptor forms a heterodimer complex with the retinoid-X receptor (RXR), a category of class II nuclear receptors. Class II receptors are known as transcription factors which regulate the expression of specific genes .In contrast to class I receptors, class II receptors are found in the nucleus of the cell. With the correspondence of Vitamin D_3 the active receptor protein complex enters the nucleus and initiates a series of molecular interactions, where the activation and suppression of specific modification of genes occurs. The specific sequences of genes that it seeks are known as the Vitamin D-responsive elements. Finally the transcription of these genes is executed and an mRNA strand is produced, where it is translated into specific proteins by ribosomes (Norman, 1998; Campbell & Reece, 2001; Holick, 2004).

Regulation: Parathyroid Hormone

Parathyroid hormone (PTH) is necessarily mentioned here on the basis that it plays a crucial role in the regulation of Vitamin D_3. The rate limiting step of Vitamin D_3 production occurs in the kidney and is accomplished by the enzyme 1α-hydroxylase. The parathyroid hormone plays an active role in directly either inhibiting or by the activation of the enzyme 1α-hydroxylase, which in turn produces l,25$(OH)_2D_3$. Also to be noted the half-life of Vitamin D_3 is only approximately 3-4 hours. Due to its short half life, its production is tightly regulated as a means of conserving it, where PTH essentially serves as this control point in the production of Vitamin D_3 (Bowen, n.d.).

Receptor Sites:

Calcitriol targets a wide range of tissues. Until recently VDR sites for l,25$(OH)_2D_3$ were only known to be located on bones, intestines and in the kidneys. New research formulates that VDR receptors are localized in cancer cell lines, the pituitary gland, the skin, hematopoietic cells, leukocytes including T and B lymphocytes, reproductive organs, ß-islet cells of the pancreas, and nerve cells. This suggests the additional roles and involvement of Vitamin D_3 , in the functioning and regulation of the immune system (Holick, 2004; Norman, 1990).

Roles:

Vitamin D_3 has been recognized for maintaining adequate levels of calcium in the serum. Additionally, it has been associated with keeping bones strong, as we know drinking milk as a source of Vitamin D_3, has been highly encouraged over the past years by the statement "Got Milk". Vitamin D_3 has been evident in preventing rickets in children and osteoporosis (osteomalacia) in adults. Moreover, recent evidence has established that Vitamin D_3 has extraordinary properties that go beyond strong bones, to such an extent that it has been reputed as a preventive medicine. It is apparent that it functions and is highly involved in the immunological system. It has been proven to prevent an extensive range of diseases including, multiple sclerosis, cardiovascular disease, diabetes, and most forms of cancer (*Vitamin D Science*, n.d.; Zittermann, 2003).

Calcium

Calcium (Ca^{2+}), a key element required for the human survival. A minor drop in calcium levels can be fatal and lead to cardiac arrest. Calcium is associated with maintaining and keeping the strength and rigidity of bones. Additionally, it is also responsible for the propagation of action potentials across nerve synapses. Release of neurotransmitters (exocytosis) into the synaptic cleft is prompted by calcium ions, which consecutively are transmitted along and received by adjacent dendritic cells in the post synaptic region. In addition, calcium plays a pivotal role in muscle contraction such as of the skeletal and cardiac muscles (Tortora, Grabowski, & Roesch, 1996, p. 125).

One of the essential attributions of Vitamin D_3 is for promoting calcium absorption. One way this is achieved is by the absorption of calcium from the lumen of the small intestine, primarily in the duodenum and jejunum. Calcitriol activates specific gene factors in DNA that translate into carrier proteins known as Vitamin D-dependent calcium binding proteins .These act as a vector for transporting proteins across the small intestine and into the blood. The most commonly known of these calcium transporters is calbindin (Norman, 1990; Tortora et al., 1996, pp. 525-527). Calcium absorption also occurs in the distal convoluted tubule (DCT) of the nephron. In concert with parathyroid hormone, production of $1,25(OH)_2D_3$ results in changes in gene expression that eventually results in the re reabsorption of Ca^{2+}in the tubule (Lajeunesse & Brunette, 1991).

A drop in serum calcium levels (normal reference range is 8.5 - 10.5 mg/dL), induces the parathyroid gland to secrete parathyroid hormone (PTH) in an attempt to restore normal calcium levels back to normal. PTH brings about the release of calcium from the bones by osteoclast formation, thereby reducing bone density. Excess PTH can lead to fragile bones, a condition known as osteoporosis. However, when Vitamin D is present, calcium is obtained by the alternative means such discussed above, conserving bone density by leaving bones intact and strong. Additionally, Vitamin D has been shown to interact with PTH, activating osteoclasts and subsequently the release of calcium. However, Vitamin D stimulates intestinal calcium uptake and excess calcium is restored to bones by osteoblast formation. Therefore, this accounts for a net gain of calcium, strengthening and maintaining the bone matrix (Tortora et al., 1996).

Cellular Differentiation

All cells originate from pluripotent stem cells, as they divide and mature they reach a climax state, where differentiation isn't possible anymore. In contrast to differentiated cells, immature cells proliferate rapidly, as evident in embryos and infants. Alternatively, aging accounts for the

lack of stem cells, brought about by terminal differentiation (*Cell Differentiation*, n.d; Oberley et al., 1980). Being that cancer cells are similar to embryonic tissues, and are relatively undifferentiated, they have a high growth rate potential (Sell, 2004). Furthermore, stem cells are present in malignant tumors, this evidently substantiates their similarity in their differentiation state and growth pattern (Farkas et al., 2009; Kopper & Hajdú, 2004; *Tumor Stem Cells*, n.d).

Vitamin D_3 via gene expression factors induces the differentiation of osteoblasts. Osteoblasts, derived from osteoprogenitor cells, secrete collagen and other essential organic components required in bone formation and maintenance. Additionally, many genes are directly involved in the cell cycle and influence proliferation, differentiation, and apoptosis in distinctively every cell. This speculates that Vitamin D_3 deficiency can consequently result in a low degree or lack of cellular differentiation (Kadakia et al., 2010; Samuel & Sitrin, 2008; Tortora et al., 1996, p. 144).

Immunology

Vitamin D_3 plays an exceptional role in the immune system. Vitamin D deficiency is an unrecognized epidemic among both children and adults in the United States. Vitamin D deficiency not only accounts for rickets among children and osteoporosis among adults but is also highly associated with increased risks for Multiple Sclerosis (MS), malignant cancers, cardiovascular disease, and both types of diabetes mellitus but primarily type 1 (Holick, 2004).

Multiple Sclerosis

Multiple Sclerosis, also known as MS, is a chronic autoimmune disease that affects the central nervous system (CNS). The CNS consists of the brain and the spinal cord. On a cellular level, neurons continuously transmit and receive signals, each being a minute, yet a necessary part of the intricate CNS orchestration, that culminate in the actions, sensations, thoughts and emotions that comprise the human experience. Normally, the path over which a nerve signal or action potential travel is protected by an insulation of fibrous material known as the myelin sheath or white matter. This is essential for efficient nerve conduction and propagation. In MS, the myelin sheath is eroded, which diminishes the ability of neurons to properly transmit signals.

It is believed that the loss of myelin is the result of mistaken attack of white blood cells. In MS something goes awry; white blood cells infiltrate the CNS, cross the blood brain barrier, seek out and destroy the myelin. As an ongoing inflammation of nerve tissue occurs, nerve signals are disrupted, leading to an array of symptoms; ranging from numbness or tingling to blindness and paralysis (*Multiple Sclerosis*, n.d.).

In MS an unknown trigger activates helper T cells (CD-4), which correspondingly recognize myelin as an antigen. Once activated, helper T cells reproduce and release cytokines such as interleukin-2. Subsequently B cells and cytotoxic T cells (CD-8) become stimulated, enabling them to cross the blood brain barrier. Once in the cerebrospinal fluid (CSF), B-cells produce immunoglobulins or antibodies that bind to myelin and oligodendracytes (cells of CNS that synthesis myelin). Cytotoxic T cells and macrophages are involved in the destruction of myelin. (Hellings, 2008)

Currently about 350,000 people in the United States and over 2 million people worldwide are affected by MS (Dangond, n.d.). Statistics have revealed that there is a higher incidence of MS in regions where inadequate sunlight exists. As a trend, the risk of developing multiple sclerosis usually increases with the distance from the equator (Figure 2). Astonishingly, regions near the equator are risk free or have a 0 MS risk coincidence. The risk of acquiring MS increases dramatically with latitude and is inversely related to sunlight. For instance, in Canada 240 out of 100,000 people have MS, compared to a mere of 9 in Mexico (*Atlas of MS Database*, n.d.). In the United States, the prevalence rate of MS below the 37th parallel is 57 to 78 out of 100,000 people. On the other hand, above the 37th parallel, the prevalence rate of MS is 110 to 140 per 100,000 people, approximately twice the rate as below the 37th parallel. An insufficient level of Vitamin D transpires under low sunlight conditions, which make one more susceptible to MS (*Nursing Home Care*, n.d.).

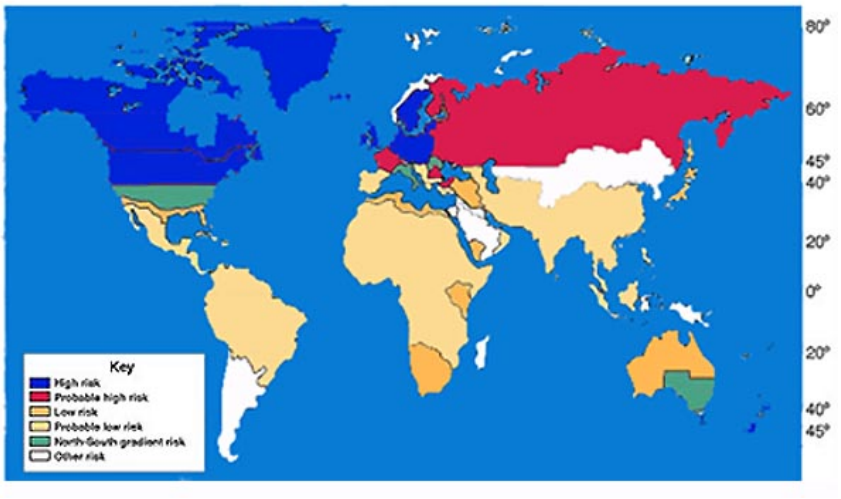

Figure 2. Epidemiology of Multiple Sclerosis: World map showing that risk (incidence) for MS increases with greater distance from the equator. Source: Multiple Sclerosis International Federation. (*Atlas of MS Database*, n.d).

In a case conducted study, a group of researchers at the Harvard School of Public Health accessed serum samples of more than 7 million military personnel. Two hundred and fifty seven of them were diagnosed with MS and had blood samples taken prior to diagnoses. Each had their serum level compared to at least two other control subjects that were disease free. They were matched on the basis of common characteristics such as age, serum collection date etc. They were then ranked into five groups based on their Vitamin D levels. Predictability, those with MS fell disproportionately into the lowest-ranking groups. Those in the highest compared to the lowest-ranking group, had a lower risk of developing MS by a staggering rate of 62 percent. (Munger et al., 2006)

Based on a study, pregnant women are encouraged to obtain Vitamin D. In this study the mothers of approximately 35,000 nurses provided information regarding their diet during pregnancy. After a span of 16 years, 199 out of these nurses developed MS. It was concluded that the mothers that consumed Vitamin D during pregnancy, gave birth to daughters with a 45 percent less risk of developing MS in years to come (Langer-Gould et al., 2010). Additionally, babies born in northern countries during the month of

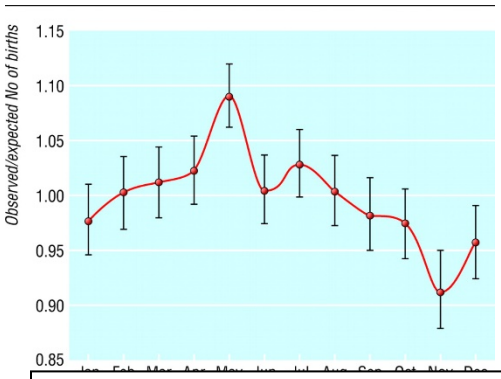

Figure 3. Pooled analysis of observed births in people with multiple sclerosis. Source: British Medical Journal. (Willer et al., 2005)

November have shown a decreased risk of developing multiple sclerosis later on in life (Figure 3). This study was based on 42,000 people and those that were born in November had a 19% decreased risk of developing multiple sclerosis over those that were born in May. Mothers pregnant during sunny months have higher levels of Vitamin D in their serum, which apparently is gets passed on to their developing baby (Willer et al., 2005).

MS patients often experience and/or are prone to bone fractures. MS is also associated with a high frequency of osteoporosis. The bone mass density (BMD) of the lumbar spine and femoral neck was found to be one to twofold lower in women with MS against controls. Low circulating levels of Vitamin D that were found in MS patients, can account for this direct relationship. (Siokaa et al., 2009; Van der Mei et al., 2007; Correale et al., 2009; Nieves et al., 1994) .Additionally, MS patients with a high Vitamin D level has shown a lesser relapse rate than controls. A dosage of 15 ng per mL, which is equivalent to 2000 international units (IU) has been shown to cut the relapse rate by half (Correale et al., 2009; Mowry et al., 2010).

Cancer

In between 1970 to 1994, cancer mortality rates in the US were approximately twice as high in the northeast compared with the southwest. An examination of 506 regions found a close inverse relationship between cancer mortality rates and levels of sunlight in the form of UV-B radiation. Annually, 21,700 premature deaths result due to a lack of Vitamin D. These results were obtained by comparing UV-B data in various regions and by using regression analysis based methods (Figure 4). This study surveyed and concluded that Vitamin D is associated with the prevention of most forms of cancers, including breast, colon, ovarian, prostate, lung, pancreatic, stomach, non-Hodgkin lymphoma, et cetera (Grant, 2002; Reichrath, 2008).

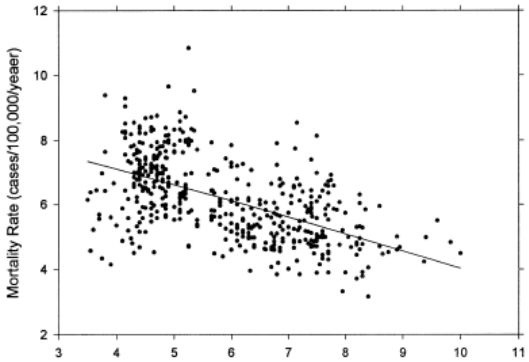

Figure 4. Annual mortality rates for breast carcinoma in white females (1970-1994) versus DNA-weighted ultraviolet (UV) radiation for July 1992. Source: American Cancer Society (Grant, 2002)

A four year randomized study conducted by the Creighton University School of Medicine revealed that Vitamin D can reduce cancer risks by a dramatic 60 to 77 percent. The subjects were 1,179 randomly selected healthy women over the age of 55. Participants were free of known cancers for a minimum of ten years prior to entering the study. Each group was either given 1,400-1,500 mg supplemental calcium, 1,400-1,500 mg supplemental calcium plus 1,100 IU of Vitamin D_3 (nearly three times the Recommended Daily Amount) or just placebos. Over the course of four years there was no statistically significant difference in cancer incidence between participants taking placebos and those just taking calcium supplements. However, those in the Vitamin D_3/Calcium group demonstrated a remarkable 60 percent cancer risk reduction, than to the placebo group alone.

On the premise that some women entered the study with undiagnosed cancers, the first year was eliminated from the study. During the last three years, the results became even more apparent in signifying the effects of Vitamin D_3 on Cancer. In those three years alone, the calcium/Vitamin D3 group exhibited a startling 77 percent cancer-risk reduction. These results were based on the

notion that calcium intervention had no significant difference in cancer reduction and were primarily due to the positive effects of Vitamin D_3. The significance of this study was that it was population based and subjects were randomly selected. Additionally, the study had a low dropout rate of less than 3.5 percent, due to the high level of treatment adherence, which applauds to the positive effects of Vitamin D_3. This provides strong evidence that Vitamin D_3 is the most effective medicine against cancer, far more than any other drug (Lappe, 2007).

There has been some controversy, however, in the role of Vitamin D_3 in the prevention of cancer. In a study conducted between 1995 and 2005, the Women's Health Initiative (WHI) gathered over 36 thousand participants, making it the largest randomized clinical trial of calcium and Vitamin D ever done. The subjects were divided into two groups and were administered twice daily either, 500 mg of calcium plus 200 IU Vitamin D_3 or just placebo alone. Participants were required to make clinical visits annually and answer a series of questions every six months, in order to determine the effects of the pills. Over an average span of seven years, a total of 322 women were diagnosed with invasive colorectal cancer. In comparison to participants who took the active Ca-D supplements with those who took placebo pills, there was no substantial difference in the rate of colorectal cancer diagnoses, prompting evidence that Vitamin D_3 may not play a role in cancer prevention (Wactawski-Wende et al., 2006).

However there are many flaws in this study, considering scientists' estimate that it may take at least 10 to 20 years for colorectal cancer to develop and the study was limited to an average of 7 years. Additionally, the WHI based its report on colorectal cancer incidence but failed to account for the many other predominant forms of cancers. The highly significant inverse relationship between Vitamin D_3 and the risk incident for all cancers was reported in the Creighton study. Nevertheless, it was observed in the WHI study that the participants with lowest quartile of serum had an overall colorectal cancer incidence that was 253 percent of the incidence in the highest quartile. Furthermore, a much lower dose of Vitamin D3 was used in the study than in the Creighton study. A dose of 400 IU was administered, which is inadequate in promoting the health benefits of Vitamin D_3 to the full potential. It is now generally recommended that 1000 IU of Vitamin D_3 daily is necessary to attain proper levels in order to achieve optimal prevention of cancer and other crucial benefits such as calcium absorption and bone strength (Holick et al., 2003). Finally, all the subjects in the study had Vitamin D insufficiency prior to the start of the trial and also during the trial based on the criterion given above (Holick, 2006).

The best source of evidence that significantly demonstrates that a higher dose of 1000 IU of Vitamin D_3 is advisable for preventing colorectal cancer, besides for other cancers, is based on a Quantitative meta-analysis pool of data from an extensive range of studies that were conducted between January 1966 and December 2004. The five studies that were surveyed contained serum collections from a wide variety of healthy volunteers who were closely monitored for periods ranging from two to twenty five years. There were a total of 1,448 total participants, 535 in the pooled analysis and 913 controls. The researches established that 1000 IU of Vitamin D_3 is associated with a 50 percent, or half of a risk reduction, of developing colorectal cancer, rather than the 400 IU of D_3 that was seen in the WHI study. The results were obtained by dividing the pooled results into quintiles with median values. Odds ratios were calculated by quintile, with the lowest as the reference group. Based on the odds for each quintile of the pooled data, a dose response curve was plotted. Data were abstracted and analyzed in 2006. It has also concluded that public awareness is needed to increase the Vitamin D_3 intake of to 1000 IU per day (Holick et al., 2007; Holick et al., 2005).

To validate the effects of Vitamin D_3 deficiency and cancer, mice with lacking VDR receptor sites were assessed. Vitamin D effects are mediated through VDR receptors, a ligand-dependent transcription factor. In comparison to normal wild type mice, the VDR knockout mice exhibited accelerated growth and heavier mammary glands. This supported the hypothesis that Vitamin D_3 inhibits proliferation and induces differentiation. Additionally, this study demonstrated that VDR absent mice were more prone and sensitive to mammary tumors that were induced due to a disruption of Vitamin D_3 (Welsh, 2004). To further determine the effects of Vitamin D and cancer proliferation, two groups of mice were fed different diets. The first group was fed a Vitamin D deficient diet for three months. The other was fed the same diet but with supplemental Vitamin D for the same duration. The mice were then injected with MC-26, a colon cancer cell line. The tumors were measured daily for an interval of twenty days. The result: the Vitamin D sufficient mice had forty percent smaller tumors than the Vitamin D deficient mice. In the Vitamin D sufficient mice the expression of the mRNA for the VDR and 1α-hydroxylase were measured and found to be 37- and 6-fold higher, respectively, in comparison to the Vitamin D absent mice. These results support the notion that Vitamin D_3 inhibits the proliferation of cancer (Tangpricha et al., 2005).

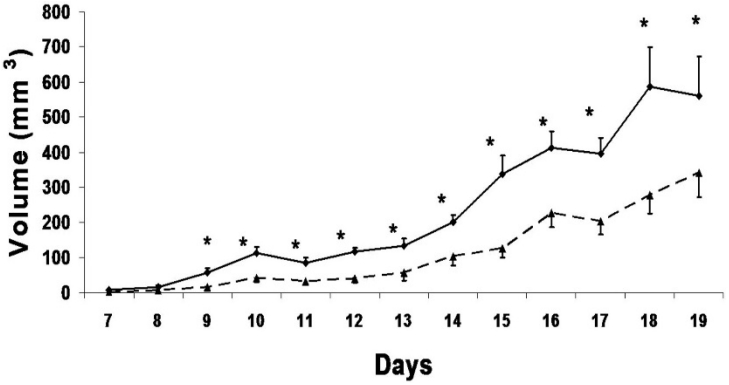

Figure 5. MC-26 tumor size in vitamin D–deficient (solid line) vs. vitamin D–sufficient (dashed line) mice.Source: Journal of Nutrition (Tangpricha et al., 2005)

Cell Cycle

Human development and maintenance requires billions of rounds of the cell cycle. The cell cycle is split into two stages, Interphase and Mitosis, respectively. Interphase accounts for approximately 95 percent of the cell cycle, making it the prominent stage in regards to cellular regulation. It consists of a sequence of three distinct phases in the order of the G_1, S, and G_2 (Figure 6). Mitosis is defined as the M phase. Depending on environmental and developmental signals, cells in the G_1 phase may temporarily or permanently cease division and enter into an arrested phase known as the G_0 resting phase .Highly differentiated cells, such as, nerve cells exist in the G_0 state (Tortora et al., 1996, p. 80) .A mishap in the cell cycle is believed to be an attribution for cancer. Simultaneously, Vitamin D is believed to be one of the crucial components responsible for cellular regulation and thus preventing cellular abnormalities that contribute for cancer.

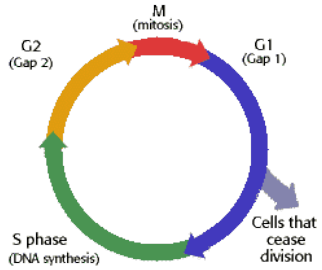

Figure 6. The cell cycle. Source: University of Arizona. (*The Cell Cycle*, n.d.).

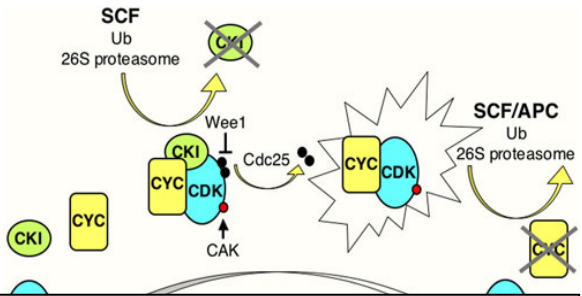

Figure 7. Model illustrating CDK regulation. Source: Harvard Medical School (Van den Heuvel, 2005).

In transition to consecutive phases, throughout the cell cycle there are several regulatory checkpoints. A group of protein kinase molecules known as Cyclin-dependent kinases (CDK's) trigger the transition of subsequent phases in the cell cycle (Figure 7). CDK's are in an active

state when bound to Cyclin proteins and by direct phosphorylation. Cyclin molecules are degraded as they progress through the cell cycle; in respect to time their concentration varies in a cyclical fashion. This ensures that cells only proceed up to the subsequent cycle. CDK's are kept in an inactive state through association with CDK-inhibitory proteins (CKIs) and by Wee1, inhibitory phosphorylation molecules. Activation requires ubiquitin-dependent proteolysis of the CKI, phosphorylation of the CDK by a CDK-activating kinase (CAK), and removal of the inhibitory phosphates by Cdc25 (**c**ell **d**ivision **c**ycle) phosphatase.

Interphase consists of several regulatory checkpoints. They ensure proper cell development, DNA replication and other critical processes that enable a cell to be error free. The G_1 checkpoint (G_1through S phase) is regulated by CDK-2, -4, and CDK6 proteins, which proper entry into the S phase. The G_2 checkpoint (G_2 to mitosis) is regulated by the CDK-1 protein, which promote M-phase progression. Identical DNA allows the cells to proceed through these checkpoints. However, the unexpected can occur, damaged DNA are often recognized and repaired. Nevertheless, unsuccessful repair attempts can result in cellular aptosis. Families of CDK kinase inhibiters (CKI) include classes of p21, p27, and p57 proteins. Their function is to inhibit a broad range of CDK's, including CDK-1, 2, 4, and CDK-6. In breast cancer cells, Vitamin D_3 impeded proliferation by preventing entry into the S-phase. Vitamin D_3 was identified to up-regulate p21 inhibiters through analogues. One such analogue is EB1089, a derivative of Vitamin D_3 with modified side chains. This targets and inactivates CDK-2 complexes, thereby terminating DNA synthesis and cell replication (Jeffrey et al., 1995; Liu et al., & Freedman, 1996; Jensen et al., 2001).

Sunscreens and Sunlight

People use sunscreen as a protective measure from the sun's rays. According to evident studies, sunscreens may do more harm than good. Sunscreens block Vitamin D production, which can contribute to far greater cancer deaths than it prevents (Dennis, Freedman, & VanBeek, 2003). Each year less than 1500 people die from skin related cancers while each day, 1500 people die from other cancers (Cancer Facts & Figures, n.d.; Skin Cancer Facts, n.d.). Sunscreens with an SPF of 8 or greater block both UVA and UVB rays (Hughes & Talbott, 2007). UVB rays are necessary to synthesize Vitamin D in the skin. Sun block may prevent skin cancer but at the same time, can be attributed to other major forms of cancer that are far more fatal.

Sunlight is often associated with promoting skin cancer but it has been demonstrated to the contrary in a San Diego study. During 1974 to 1984, there were 176 confirmed cases of melanoma that were identified in active-duty white male personnel enlisted in the US Navy. Occupations were grouped in three categories based on sunlight exposure, as indoor, outdoor or a combination of indoor/outdoor. Compared to the U.S. civilian population, those working indoor had a higher melanoma occurrence of 10.6 per 100,000 people. Those who worked in occupations that required spending time both indoors and outdoors had the lowest rate, of 7.0 per 100,000 people. Additionally, incidence rates of melanoma were higher on the trunk than on more common sunlight exposed areas, such as the head and arms. This establishes that moderate amounts of sunlight can prevent melanoma rather than cause it (Garland et al., 1990).

According to the CDC melanoma has been rising sharply for the last several decades ("Notice to Readers:," 2000). Yet, concurrently, as year's progress, less time is spent outdoors. In the early1900's more than 75% of the North American population worked outdoors compared to only 10% by the 1970's (Genius, 2006). This trend illustrates an inverse relationship between

sunlight and melanoma. Furthermore, the greatest increase of melanoma occurred after the introduction of sunscreens. Death rates in the US between the 1950's and 1990's doubled in men and nearly tripled in women (Garland et al., 1992,).

Analytic studies were performed on articles published in between 1966 to 2003 relating to melanoma and sunscreen use. Odds ratios were pooled across studies by using standard meta-analytic techniques. It was concluded however, that, no correlation was seen between melanoma and sunscreen use (Dennis, Freedman, & VanBeek, 2003). In between 1978-1992, cancer mortality rates were analyzed in Spanish provinces. The mortality rates of melanoma and other forms of cancer were significantly correlated with latitude. This concluded that melanoma occurs more frequently in northern latitudes versus lower latitude areas (Grant, 2007).

Although sun light has been proven to have positive effects, it has limits too. There is a common rule that applies to everything; "too much of a good thing is bad". There should always be a healthy balance between things. The recommended optimal daily intake of Vitamin D for an adult is approximately 1000 IU (international units). This can be achieved by spending approximately, depending on the day, about 15-20 minutes in the sun. Obviously, prolonged endurance in the sun requires one's discretion and application of sunscreen may be necessary (Vitamin D, n.d.).

Toxicity

In healthy individuals, Vitamin D blood levels are normally found in the range of 32 to 70 ng per mL. Vitamin D is a fat soluble molecule, making elimination difficult. An advantage to sunlight obtained Vitamin D is that it is automatically regulated by a negative feedback loop. An adequate threshold level of Vitamin D prompts the shutdown of further synthesis. This makes overdosing unattainable by regulating Vitamin D levels. However, Vitamin D obtained in supplemental form can pose a serious threat. Toxicity results from overdosing, which is about over 1,500 micrograms or 10,000 IU, ten times the recommended FDA daily dosage. Symptoms of overdosing include nausea, poor appetite, constipation, weakness, weight loss, confusion, heart rhythm abnormalities and Kidney stones.

Conclusion

The enormous positive effects of Vitamin D are vividly apparent. Its functions range from Calcium absorption to the suppression of tumors. A diet rich in Vitamin D is extremely encouraged. On the other hand, a deprivation of Vitamin D results in a multitude of consequences. It is no coincidence that in recent years Vitamin D emerged in the spot light of research, involving the formulation of numerous exceptional works.

Unfortunately, modern society encourages low levels of Vitamin D. People do not go out often and when they do are in cars. Pollution obscures some of the sun's beneficial rays. Furthermore, spending time in the sun is associated with significant sun block use. Sunlight shouldn't be averted; it is a benefactor to humans. As mentioned in the Bible of Genesis, when redemption will arrive, G-d will use the magnificent powers of the sun to heal the righteous.

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The Peanut Allergy Epidemic

Rivky Sachs

ABSTRACT

Peanut allergy is one of the most predominant food allergies. It accounts for majority of the highly severe and fatal allergic reactions to food. Peanut allergy is generally detected early in life and is commonly associated with other atopic disorders such as asthma, eczema, and rhinitis. The prevalence and pervasiveness of peanut allergies is increasing worldwide, and most peanut allergic patients have lifelong sensitivities to peanuts (de Leon et al, 2008).

Patients with severe allergies must stringently avoid any contact with peanuts and depend on intramuscular epinephrine (EpiPen) to counteract the reaction caused by intake of peanuts. Much research is dedicated to developing new treatments that may be able to induce tolerance in peanut allergic individuals without adverse side effects. This paper reviews the current understanding of clinical characteristics, pathogenesis, and hypothetical causes for the rise in prevalence of peanut allergies. It also discusses genetic risks and environmental effects of peanut allergy. Furthermore, it presents emergent future therapies and methods to prevent the development of peanut allergies in infants.

INTODUCTION

Allergy is defined as an adverse immune response to food (Sicherer and Sampson, 2010). Such responses can be mediated by IgE antibodies aimed at specific allergens, or they can be triggered by other cellular activities (Wood, 2009). IgE mediated reactions are immediate, and symptoms can range from acute or chronic atopic reactions to fatal anaphylaxis. Non- IgE

mediated allergy occurs gradually and is evident by chronic skin conditions and gastrointestinal discomfort (Eigenmann, 2009). Peanut allergy is an IgE mediated response.

EXPOSURE TO THE FOOD ALLERGEN

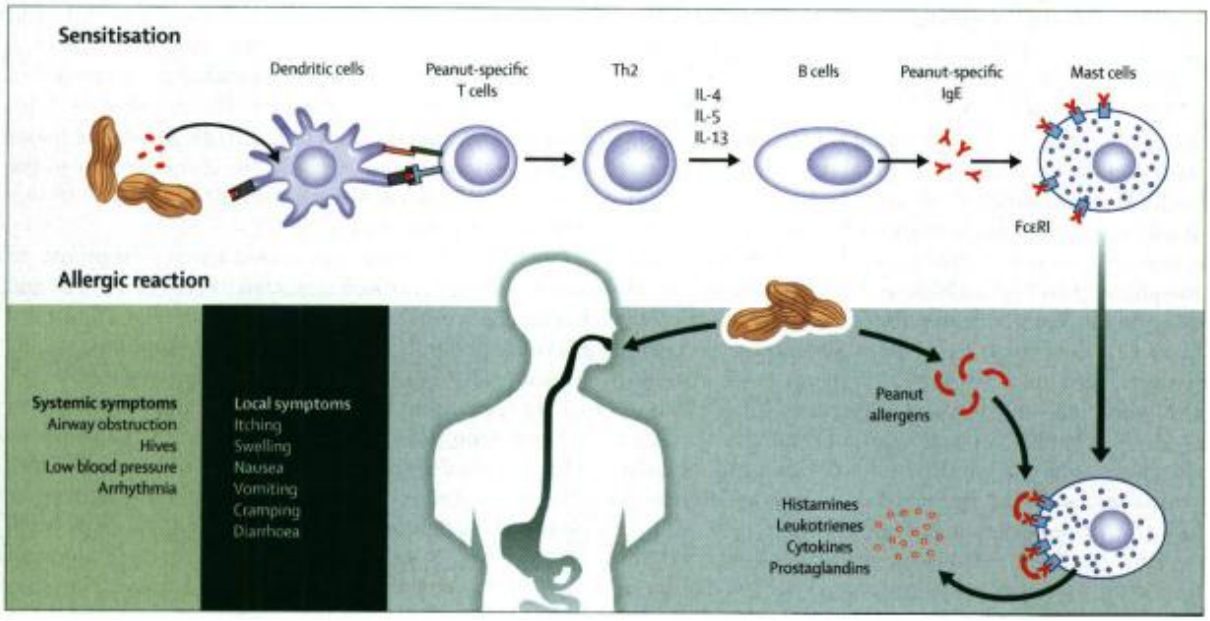
Peanut allergens are introduced to the body through one of three particular ways: ingestion, skin contact, or inhalation of airborne particles. In the most prevalent form of the allergy, peanuts must be consumed for an allergic reaction to ensue since the allergic reaction is not frequently activated by skin or air contact with the peanut allergen. Food enters the digestive system through the mouth and the food allergen is first introduced at the mucosal surface of the gastrointestinal tract (Burks, 2008).

PATHOGENESIS

At the mucosal surface of the gut, food proteins are digested by specialized epithelial cells that transfer them to antigen presenting cells such as dendritic cells which then process them into peptide fragments. These peptide fragments are presented on the cell surface by class II major histocompatibility complex (MHC) molecules. As the antigen presenting cell presents the antigen to T helper cells through MHC/T cell receptor interaction it activates the T helper cells. This process instigates humoral and cellular mediated responses that are associated with peanut allergy (de Leon et al, 2008).

In peanut allergic individuals, the activation of T helper 2 (T_H2) cells causes the production of cytokines such as interleukins including IL-4, IL-5, IL-9, and IL-13. These interleukins are released in increased amounts as compared to individuals that are not allergic to peanuts, thus exciting B cells to synthesize IgE antibodies. These peanut specific IgE antibodies are attached to mast cells in the gastrointestinal tract mucosa, skin, and respiratory tract mucosa and to basophils (Burks, 2003, Burks, 2008).

Upon ingestion of peanuts, the peanut proteins bind with the specific IgE antibodies on mast cells and basophils thereby secreting inflammatory mediators such as histamine. T_H2 cells and mast cells also produce tumour necrosis factor- *a* (TNF-*a*), IL-5, and chemokines, which cause eosiniphils to accumulate at the site inflammation. Eosiniphils stimulate the release of other inflammatory mediators which lead to clinical symptoms that will immediately develop (de Leon et al, 2008).



Allergic reaction to peanut (Burks, 2008)

CLINICAL SYMPTOMS

Clinical symptoms can be triggered by ingesting ten milligrams of peanut protein. The time it takes for symptoms to develop ranges from seconds up to two hours after ingestion. The majority of reactions of IgE mediated disorders include skin, respiratory, and gastrointestinal symptoms. Local symptoms include itching, swelling, nausea, vomiting, cramping, and diarrhea (Burks, 2008).

Anaphylaxis, a systemic symptom, can often be fatal. The early symptoms of anaphylaxis include oral and pharyngeal pruritus and chest tightness. Further symptoms of wheezing, hypotension, arrythema, and unconsciousness can develop into fatal and near fatal reactions (Lee et al, 2003). Anaphylaxis is a rapidly progressing multiple organ system reaction that can lead to cardiovascular failure. This reaction is a result of a massive release of mediators such as histamine (Sicherer and Sampson, 2010).

EPIDEMIOLOGY AND RISING PREVALENCE

Studies done in the United Kingdom and North America show peanut allergy prevalence rates in children have increased, in fact, almost doubling. Peanut allergy affects more than 1% of school aged children (Sicherer and Sampson, 2007). Grundy et al did two sequential cohorts in the Isle of Wight, United Kingdom six years apart. Children at the age of 3 and 4 received skin prick tests and those with positive results were given oral peanut challenges unless there was an otherwise convincing history of peanut allergy. The study confirmed an increase in peanut allergy from .5% to 1.0% between 1989 and 1995.

The same incidence was reported in the U.S. with rates similar to those received in the previous study. In 1997, Sicherer, Munoz-Furlong, and Sampson performed a population based, cross-sectional, random telephone survey in the United States using a standardized questionnaire. In 2003, they conducted a five year follow up study to compare the results of the prevalence

estimates. The study indicated a doubling in peanut allergy in children from 0.4% in 1997 to 0.8% in 2002.

Kagan and colleagues also confirmed the assumption of increasing prevalence of peanut allergy using diagnostic testing. They assigned questionnaires regarding peanut ingestion to parents of children in kindergarten through grade 3 in randomly selected schools in Montreal. Respondents were grouped as follows: (1) peanut tolerant, (2) never-rarely ingest peanut, (3) convincing history of peanut allergy, and (4) uncertain history of peanut allergy.

Groups 2, 3, and 4 received peanut skin prick tests (SPTs), and if responses in groups 2 and 4 were positive, measurement of peanut specific IgE was taken. Children in group 3 with a positive SPT were considered to have peanut allergy without more testing. Children in groups 2 and 4 who had peanut specific IgE levels less than 15kU/L were administered oral peanut challenges. These techniques ensured that only children who had real peanut allergic symptoms were considered sensitive. Their estimate of 1.5% proves that the prevalence of peanut allergy was even higher than what had previously been reported (Kagan et al, 2003).

However, five years later a follow up study was performed using the same methodology and population to determine whether prevalence was increasing over time. The prevalence estimate was 1.62%, indicating a .13% difference. They concluded that the prevalence has remained stable among this population. Furthermore, they refuted the studies conducted by Grundy et al and Sicherer et al by saying that the increasing prevalence estimates they reported was a result of environmental changes during the period that they experimented and that participation rates were lower at follow up, thus leading to an overestimate of prevalence. Also, they claim that the .4% difference in estimates of the case study done by Sicherer et al is not statistically significant (Ben Shoshan, et al 2003).

There are numerous problems with this follow up study done in Montreal and the conclusions drawn from its results. This study was conducted in one small region with low participation rates, as compared to the survey performed by Sicherer et al which covered the entire United States with a census of over 13,000 individuals. Therefore, .4% estimate is in actuality a large percentage of the population.

Moreover, they base their estimates on the assumption that peanut specific IgE levels of 15kU/L or greater indicate a peanut allergy. Consequently, they only presented peanut oral challenges to children who had positive SPTs with less than 15kU/L peanut specific IgE levels; even though there is no proof that a positive peanut specific IgE level implies that an individual does indeed have a peanut allergy. According to recent studies, between 50% and 75% of patients with specific IgE to food are not allergic to the food (Roberts et al 2005). On the contrary, in the study done by Grundy et al, all children with positive skin prick tests, not including those with a history of peanut allergy, underwent oral challenges regardless of their peanut specific IgE level. They did not rely on peanut specific IgE levels to determine if the child has a peanut allergy. Due to this and many other incorrect assumptions, this study loses its credibility. In addition, their criticism and disapproval of other studies is inherently biased and lacks evidence.

Over the past twenty years there has been a universal increase in children being diagnosed with this allergy (Grundy et al, 2002). The rise in prevalence of peanut allergies has

initiated much research in this field. There are numerous new hypotheses and theories to explain the apparent peanut epidemic.

SPECIFIC PEANUT ALLERGENS

Antigens that involve an IgE response are referred to as allergens, which are the glycoproteins found in food. Eight peanut allergens have been discovered and are classified as Ara h 1 to Ara h 8 (Burks 2008). The three major peanut allergens are Ara h 1, Ara h 2, and Ara h 3. Ara h 1 and Ara h 2 are known to be highly allergenic, because numerous peanut allergic individuals have serum specific IgE for these allergens.

The classification of Ara h 3 has not yet been identified: one study showed serum IgE reactivity in 44% of peanut allergic subjects while another found it in 95% of the patients. Ara h 4-7 are not as reactive with IgE in patient sera and have not yet been classified. Ara h 8 is different than the others as it is established to be a major allergen in Central Europe in individuals who display a co-allergy to peanut and birch pollen (de Leon et al, 2008).

BIOCHEMICAL PROPERTIES OF PEANUT ALLERGENS

According to Koppelman et al, many peanut allergens have specific physiochemical properties that display resistance to digestive enzymes and heat processing. Their study proves that Ara h1 is heat stable, although its structure is changed significantly when heated. The resistance of some peanut allergens to heat and enzymes may be connected to their ability of forming stable dimer and trimer complexes. This enables the peanut proteins to reach the intestinal mucosa intact, thus increasing their allergenicity and effectiveness.

MAILLARD REACTION

A major reaction that happens during the browning or processing of foods is the Maillard reaction. One of the main chemical modifications that peanut proteins undergo is the Maillard reaction which reduces sugars and primary amine groups thus increasing the allergic properties of peanuts. Maleki, Chung, Champagne, and Raufman proved this by conducting an in vitro model copying the Maillard reaction. Ara h 1 heated in the presence of sugars exhibited increased allergenicity, while Ara h 1 heated without sugars degraded and was barely discernible. Roasted peanut extracts were found to bind to IgE serum of allergic individuals at about 90- fold higher than raw peanuts. The Maillard reaction contributed to this effect. This study supports the findings of Nordlee et al that roasted peanuts bind IgE at higher levels than raw peanuts. It also shows that modification of proteins during the roasting process increases the IgE binding sites and enhances other allergenic properties in peanuts (Maleki et al, 2000).

EFFECTS OF COOKING METHODS ON PEANUT PROTIENS

There is a much lower prevalence of peanut allergy in China than in the United States, even though there is a higher rate of peanut consumption in China. Peanuts are fried and boiled in China; whereas they are usually roasted in the U.S. In 2001, Beyer et al conducted a study to examine whether the method of preparing peanuts could be a contributing factor to the difference of allergy prevalence between China and the United States. The Chinese-American population seems to have a similar prevalence of peanut allergy as the general U.S. population, so genes cannot account for this difference.

Two kinds of peanuts grown in the United States were roasted, boiled, and fried. Proteins were examined using SDS-PAGE and immunoblotting. Reactions to the peanuts were compared by using immunolabeling with sera from 8 individuals with peanut allergy. Frying and boiling modified the proteins of both types of peanuts in a similar way. The roasted peanuts had more Ara h1 which resulted in an increase in IgE binding intensity as compared to the boiled and fried peanuts. Furthermore, in the fried and boiled peanuts there was less IgE binding to Ara h 2 and Ara h 3 than there was in the roasted peanuts.

Beyer et al concluded that frying and boiling peanuts, as done in China, reduces the allergenic property of peanuts as compared to the roasting method which is widely used in the United States. Roasting uses higher temperatures that probably strengthen the allergenicity of peanut proteins by causing permanent changes in the structure and may account for the difference in prevalence of peanut allergy in the two countries (Beyer et al, 2001).

Although this study has strong supporting data, the tests performed are statistically insignificant and do not prove its hypothesis. There were only 8 samples of patient sera taken to compare the different reactions. This is a very small range with little diversity, and it does not differentiate between age groups. Recent statistics confirm that there are in fact more children with peanut allergies in China than what was initially thought (Burks, 2008). This does not invalidate the studies that show that roasting enhances peanut allergenicity. More information is needed about the prevalence of peanut allergy to better understand this subject matter.

ARA H 2 FUNCTIONS AS A TRYPSIN INHIBITOR

In their previous study, Maleki et al showed that the allergenicity of peanut proteins is enhanced due to thermal processing. Because of the increasing prevalence of peanut allergy and the severity of the symptoms, another study was performed by Maleki and his colleagues to find out whether any specific functions are associated with the major peanut allergen, Ara h 2, and whether these functions are affected by processing. Maleki et al conducted a protein domain homology search to figure out the functions of Ara h 2. Reputed functions were tested via enzyme assays and protein gel electrophoresis. Afterward, the structural properties of Ara h 2 from purified and roasted peanuts were compared.

According to their results, Ara h 2 purified from peanuts acts as a trypsin (digestive protein) inhibitor and roasting increases the trypsin inhibitory activity by a 3.6-fold. Ara h 2 was also found to protect Ara h 1, a second major allergen, from trypsin digestion. This characteristic was improved in Ara h 2 purified from roasted peanuts.

The data indicates that thermal processing may play a significant role in increased allergenicity of peanuts. Not only does roasting change the structure and stabilize peanut proteins as has already been proven, but it also alters its functional properties which further contribute to its increased allergenicity. Ara h 2 increases its resistance toward and provides Ara h 1 with extra protection against trypsin digestive enzymes (Maleki et al, 2003).

GENETIC RISK FACTORS OF PEANUT ALLERGY

It is implausible that genetic factors contributed to the increase of peanut allergy in certain parts of the world over the past decade. Nonetheless, it is probable there are genetic predisposing factors that contribute to the development of food allergy, just like there are genetic factors associated with other atopic diseases such as asthma and eczema. More research is

needed to figure out which genetic polymorphisms are associated with specific allergies (Lack, 2008).

Peanut allergy has been associated with familial inheritance and genetics. Hourihane, Dean, and Warner calculated rates of other atopic symptoms in people with peanut allergy and the prevalence of the allergy in their families. They concluded that a child with a peanut allergic sibling has a 7- fold increase of developing a peanut allergy (Hourihane et al, 1996).

A study was performed by Sicherer et al to determine if genetic factors influence peanut allergy by comparing the rate of this allergy among monozygotic and dizygotic twins. The method relies on the fact that monozygotic twins share all of the same genes, while dizygotic twins share only half of their genes. They found that there was a considerably higher peanut allergy association among monozygotic twin pairs than there was among dizygotic twin pairs. In monozygotic twins, a child has a 64.3% chance of developing peanut allergy if his/her twin has the allergy, while a dizygotic twin has only a 6.8% chance.

This data suggests that specific genes influence the development of peanut allergy in an individual. However, there are limitations to the results received in Sicherer et al's study. Firstly, it was impossible to get a population based sample, because of the low population rate of twins with peanut allergies. In addition, environmental factors affect gene expression and are also responsible for the increasing prevalence of peanut allergy.

VITAMIN D HYPTHOSES

The vitamin D hypotheses have been developed to explain the increase of allergies and asthma, and it involves two opposing studies. One is the vitamin D excess hypothesis which proposes that increased Vitamin D level results in a higher prevalence of allergies. The vitamin D deficiency hypothesis argues the opposite. Wjst, the first to work out the vitamin D excess hypothesis, recognized that in German farming communities there was less vitamin D supplementation in food and less allergies found in children. The theory was further advanced when they found an increase in allergies in Bavaria which happened around the same time that vitamin D supplementations were added to children's diets to prevent rickets. Milner et al and Hypponnen et al both performed studies which proved that infants who had vitamin D added to their diets were at a greater risk for developing food allergies.

Those that support the vitamin D deficiency hypothesis say that people who do not get enough vitamin D from sunlight have a greater prevalence of allergies. They proved that countries further from the equator are less exposed to sunlight yet have higher rates of asthma (Lack, 2008). One intriguing study performed by Camargo et al provides evidence for the vitamin D deficiency hypothesis. They observed a strong north-south gradient for the prescription of EpiPens in the United States. In the New England region 8-12 EpiPens per 1000 persons were being distributed, while the southern states had only 3 EpiPen prescriptions per 1000 persons. Their data supports the presumable association between low vitamin D rates and allergies. There are proofs to substantiate both of these hypotheses, yet the vitamin D controversy remains unresolved.

ORAL AND NONORAL EXPOSURE TO PEANUT ALLERGEN

Infants can be exposed to peanuts through ingestion or via environmental exposure such as topical use of peanut derivatives. Other types of exposure can occur in utero or through breast

milk (de Leon et al, 2007). The exposure route through which sensitization occurs remains unknown, and there are various schools of thought on this subject (Fox et al, 2009).

PEANUT AVOIDANCE DURING PREGNANCY, LACTATION, AND INFANCY

In order to reduce the prevalence of peanut allergy, the UK health department advised pregnant or nursing mothers to avoid eating peanut products if they themselves or other immediate family members of the fetus are atopic (Ewan, 1998). Hourihane et al conducted a study five years after the UK peanut avoidance advice which showed that the prevalence of peanut allergy in the UK increased. The authors concluded that the government's advice showed no significant influence on the outcome (Hourihane et al, 2007).

Similarly, the American Academy of Pediatrics committee recommended avoiding peanuts in the infant's diet during the first three years of life in order to prevent peanut allergy development (Baker et al, 2000). It was later confirmed that peanut allergy in children doubled since the recommendation was put into effect (Burks, 2009). However, it still remains tentative whether peanut avoidance during pregnancy and lactation has a positive, negative or no impact at all on the prevalence of this allergy in children (Hourihane et al, 2007).

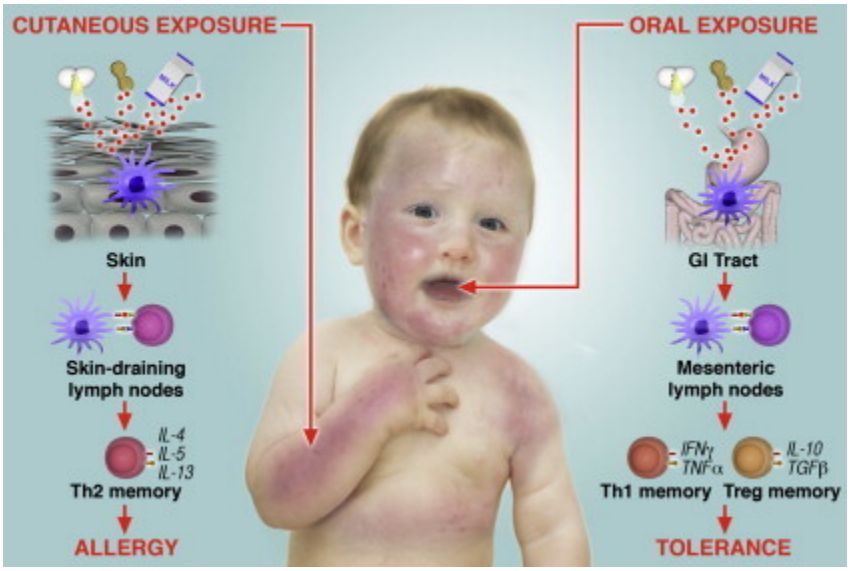
DUAL-ALLERGEN EXPOSURE HYPOTHESIS

Fox et al presented data substantiating a different theory in which low-dose early environmental exposure increases the probability of developing a peanut allergy as opposed to maternal consumption of peanuts. Since most children with peanut allergy react on their first oral exposure to peanut, they hypothesized that cutaneous exposure is the route through which allergic sensitization occurs. In a previous study, they showed that topical exposure to creams containing peanut oil was a risk factor for the development of peanut allergy. Almost 91% of the children with peanut allergy were exposed via use of peanut oil in the first six months of life. Moreover, in households where peanut is consumed a lot, there are other forms of environmental exposure that can occur such as cutaneous contact or inhalation of the allergen. For example, there is always remaining peanut allergen on the hands or in the saliva of someone who consumed peanuts. When an individual ingests peanuts and touches or kisses someone not yet exposed, it can cause sensitization.

At first, it seems that the results of the study demonstrate a strong association between increased maternal consumption of peanuts during pregnancy and lactation and children who develop peanut allergy. This inconsistency was resolved by adjusting for household peanut using logistic regression. They concluded that maternal consumption is irrelevant, since mothers in households with high peanut consumption are more probable to eat peanut because of its accessibility.

One group of children had high levels of environmental exposure and consumption of peanuts during infancy, yet they were tolerant to peanuts. This data supports the hypothesis that infant oral exposure to peanuts can induce tolerance and prevent development of peanut allergy even where there is environmental exposure as well. This study demonstrates that high levels of environmental exposure due to household consumption increases cutaneous sensitization to peanuts. It also refutes the original hypothesis that peanut consumption during pregnancy and lactation causes the development of peanut allergy in infants (Fox et al, 2009).

This theory is known as the dual-allergen exposure hypothesis. Low-dose early environmental exposure of peanut is taken up by Langerhan's cells in the skin which leads to T_H2 responses and release of IgE by B cells resulting in allergic sensitization. On the contrary, early infant consumption causes T_H1 and regulatory T cell responses thereby inducing tolerance. This hypothesis reveals the connection between the presence of infant eczema and the ensuing development of food allergy. It also explains the rates of food allergies in different parts of the world and changes over time (Lack, 2008).



(Lack, 2008)

EARLY CONSUMPTION OF PEANUT

In order to investigate the effect of early consumption of peanut, Du Toit et al, compared peanut allergy prevalence among Jewish children in the UK and Israel. Israeli children consume peanut in high quantities early in life, while UK Jewish children avoid it altogether. Nonetheless, Jewish children in the UK had a peanut allergy prevalence of more than 10-fold higher than in Israel. Their findings imply that early and frequent high dose peanut consumption may thwart the development of peanut allergy (Du Toit et al, 2008). Furthermore, studies suggest that people in African and Asian countries, where the peanut allergy rate is relatively low, consume peanuts throughout pregnancy and infancy. Conversely, in the U.S., U.K., Australia and Canada peanut consumption is higher, yet there is a much greater prevalence of peanut allergy. This is due to the fact that there is a lot of environmental exposure accompanied by avoidance of peanuts during infancy (Lack, 2008).

Food allergies among allergy clinic patients

Country	Peanut allergy (%)	Dietary practice recommendations (infant peanut consumption)

Country	Peanut allergy (%)	Dietary practice recommendations (infant peanut consumption)
United Kingdom (n = 191)	25	Avoidance
United States (n = 300)	69	Avoidance
Israel (n = 992)	2.1	High infant consumption
Philippines (n = 184)	0	High infant consumption

(Lack, 2008)

The results from these studies suggest a possibility that high levels of environmental exposure without oral exposure can result in peanut allergy, while elevated peanut ingestion can induce peanut tolerance. Further study is needed to investigate the validity of this hypothesis. It is extremely difficult to differentiate between the precise effects of maternal consumption and household peanut exposure. Moreover, there are other factors that can explain the difference in peanut allergy prevalence between Israel and England.

First, there may be a delayed increase in Israel since the prevalence of 0.17% that Du Toit et al recorded is a 4- fold increase from what was originally recorded by Dalal et al in 2002. Additionally, most peanuts in Israel are boiled which decreases its allergic properties as compared to being roasted which increases its allergenicity. Nonetheless, this promising theory is being investigated further by the Learning Early About Peanut Allergy study through a randomized controlled trial to assess whether early peanut consumption in high risk infants will prevent peanut allergy more effectively than avoidance during infancy (Burks, 2009).

FUTURE THERAPIES

Most of the clinical studies being performed on peanut allergy are devoted to finding an effective treatment to help patients with severe reactions to peanuts (de Leon et al, 2007). The standard subcutaneous immunotherapy has been eliminated because it resulted in many adverse reactions. Immunotherapeutic approaches to food allergy in general, have recently been categorized as food allergen-specific and food allergen-nonspecific. Presently, there is no cure for food allergy. The only treatment for peanut allergy is stringent avoidance of all peanut containing products. The immunotherapeutic approaches discussed below are tentative and require further research (Sicherer and Sampson, 2007).

SUBLINGUAL AND ORAL IMMUNOTHERAPY

Sublingual immunotherapy (SLIT) and oral immunotherapy (OIT) are both allergen specific therapeutic approaches. Both these therapies are designed based on the theory of induced tolerance when an antigen is presented at the oral mucosa/ gut associated lymphoid system. In SLIT and OIT, patients are introduced to small amounts of the allergen orally and the amount is increased over time. Although these therapies do provide desensitization for many patients on therapy, there is no proof that induces tolerance. Furthermore, there are risks of extreme side

effects and anaphylaxis if the patient stops therapy for 1-3 weeks and then resumes with the same dosage (Sicherer and Sampso, 2007).

MODIFIED PROTEIN VACCINE

The engineered recombinant protein strategy is an allergen-specific method which attempts to minimize IgE activation by mutating IgE binding sites. The three major peanut allergens are separated to identify their allergenic epitopes/ IgE binding sites (Nowak-Wegrzyn et al, 2009).Then, mutations are made to the peanut allergen gene which makes these sites nonreactive to IgE. When the mutated peanut allergen is expressed, it will result in hypoallergenic variants which can be used for immunotherapy (de Leon et al, 2007). This method is much safer than standard subcutaneous which injected the native protein into the skin. This approach looks very promising in murine models and human studies are being planned for further testing (Sicherer et al, 2010).

CHINESE HERBAL MEDICINE

Li and colleagues developed a 9-herb preparation known as Food Allergy Herbal Formula (FAHF-2), which is an allergen-nonspecific approach that prevents peanut induced-anaphylaxis (Sicherer and Sampson, 2007). In one experiment, peanut allergic mice treated with FAHF-2 for 7 weeks were challenged 1, 3, or 5 weeks after therapy. They recorded that IgE levels were particularly reduced by FAHF-2 and remained that way as long as 5 weeks after therapy was completed. This result seems to be connected to the suppression of T_H2 cytokines by the FAHF-2. The full protection that FAHF-2 demonstrated was replicated in many experiments and always showed a consistent response. This herbal formula might prove to be a valuable and harmless treatment for peanut allergy (Srivastava et al, 2005)

ADDITIONAL IMMUNOTHERAPEUTIC METHODS

Other types of food allergen-specific therapies include cytokine-modulated immunotherapy, immunostimulatory sequence-conjugated protein-modulated immunotherapy, plasmid DNA-based immunotherapy, and allergen peptide immunotherapy. All these treatments try to lessen the T_H2 response or induce tolerance to a specific food allergen (Burks, 2008). One study shows that similar proteins found in soybeans can be used for immunotherapy to desensitize peanut allergic mice (Pons et al, 2004). Another allergen non-specific approach is anti IgE therapy which does not cure the allergy but rather reduces fatal reactions in patients (Burks, 2008). The responses to this therapy turned out to be inconsistent, and this method has been suspended due to safety issues (Nowak-Wegrzyn et al, 2009).

Selected immunotherapeutic strategies:

Therapy	Immune rationale	Benefits	Observations to date
Standard subcutaneous immunotherapy (native allergens)	Antigen presentation in nonmucosal site results in T_H1 skewing	Proved for venom and respiratory allergy, possible benefit (pollen) for oral allergy syndrome	Primarily avoided for risk of anaphylaxis (eg, peanut)
Sublingual/OIT	Antigen presentation to mucosal site provides desensitization and	Natural foods, reduced risk of systemic anaphylaxis compared with injections	Mounting evidence for desensitization and relative safety; unclear effect on

Therapy	Immune rationale	Benefits	Observations to date
	might induce tolerance		tolerance
Modified protein vaccine	Reduced IgE activation by mutation of IgE-binding epitopes	A safer form of immunotherapy compared with injection of native protein	Murine models show promise, human studies are planned
Peptide vaccine (overlapping peptides)	Peptides are less likely to cross-link IgE, avoiding mast cell activation	No requirement for IgE epitope mapping/mutation	Limited
Conjugation of immune stimulatory sequences to allergen and additional adjuvant methods	Enhance T_H2 response by activating innate immune receptors (using specific sequences or whole bacteria)	Increased efficacy, possibly improved safety	Preclinical studies
Plasmid DNA-encoded vaccines	Endogenous production of allergen might result in tolerance	Possible 1-dose treatment	Murine models reveal strain-specific response
Anti-IgE antibodies	Targeted toward Fc portion of antibody, can inactivate IgE with reduced risk for activating mast cells	Not food specific Some response in eosinophilic gastroenteropathy (pilot study)	Preliminary study showed improved threshold overall but did not show uniform protection
Chinese herbal medicine	Mechanism unknown	Not food specific	Murine models show efficacy Human safety studies are underway
Cytokine/anti-cytokine (eg, anti–IL-5)	To interrupt inflammatory signals	Might allow directed interruption of inflammatory processes without need for food restriction	Preliminary study shows benefit for eosinophilic esophagitis.

(Sicherer et al, 2010)

CONCLUSION

It has been proven that peanut allergy is becoming increasingly prevalent and poses a health threat worldwide, specifically in developed countries. In peanut allergic individuals, the exposure of peanut allergens via the gut, skin, or air can lead to clinical symptoms ranging from mild skin conditions to fatal anaphylaxis. Much research has been done to investigate the immunologic, environmental, and genetic affects on the development of peanut allergy. Studies

show that peanut proteins undergo the Maillard reaction during thermal processing, thus increasing the allergenicity of peanuts. Furthermore, roasting uses elevated temperatures that strengthen the allergic properties of peanut proteins by causing permanent changes in its structure. Another study suggests that Ara h 2 purified from peanuts acts as a trypsin inhibitor and roasting increases the trypsin inhibitory activity.

Some studies propose that genetic factors are linked to the development of peanut allergy, while the vitamin D hypotheses suggest that either increased or decreased levels of vitamin D leads to increasing allergy prevalence. Furthermore, it was originally thought that avoidance of peanuts during pregnancy and lactation can prevent development of peanut allergy in the fetus/infant. However, this was disproven, and the dual allergen exposure hypothesis, which states that low-dose early environmental exposure increases the probability of developing a peanut allergy, is the newest proposition. There are some allergen specific and allergen nonspecific therapies available to reduce fatal peanut allergic reactions. Research is being done to provide a therapy that induces tolerance to peanut without adverse side effects.

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