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LYMPHOCYTES IN ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

Alan P. Knutsen

Divisions of Allergy and Immunology, Saint Louis University Health Sciences Center, 3662 Park Avenue, St. Louis, MO 63110

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1. ABSTRACT

Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity lung disease mediated by an allergic late-phase inflammatory response to *Aspergillus fumigatus* antigens. ABPA is characterized by markedly elevated *Aspergillus*-specific and total IgE levels and eosinophilia, and manifested by wheezing, pulmonary infiltrates and bronchiectasis and fibrosis, which affect asthmatic and cystic fibrosis (CF) patients. In the pathogenesis of ABPA, *A. fumigatus* proteases play a role in facilitation of antigen transport across the epithelial cell layer by damaging the epithelial integrity and by a direct interaction with epithelial cell surface receptors, resulting in pro-inflammatory cytokine production and corresponding inflammatory responses. In genetically susceptible asthmatic and CF patients, this leads to an allergic inflammatory response to *Aspergillus* allergens. A genetic susceptibility is HLA-DR restriction demonstrated by increased frequency of HLA-DR2 and/or DR5 and lack HLA-DQ2. IL-4 plays a central role in the development of allergic inflammatory responses. Our group has demonstrated that ABPA patients have increased sensitivity to IL-4 stimulation and skewing of Th2 responses to *Aspergillus* allergens in ABPA subjects and a Th1 response in non-ABPA subjects. Interestingly, *Aspergillus* allergens stimulate IL-10 synthesis is both ABPA and non-ABPA subjects.

2. INTRODUCTION

Allergic bronchopulmonary aspergillosis (ABPA) is a late-phase allergic inflammatory response to *Aspergillus fumigatus* (Af) allergens that occurs in genetically susceptible patients with asthma and cystic fibrosis (1-33). In addition, Af allergens, especially proteases, have direct effects on the respiratory epithelium that result in pro-inflammatory responses and increased allergen exposure to the pulmonary immune system. The allergic inflammation to Af leads to pulmonary central bronchiectasis and fibrosis. Thus, the understanding of the pathogenesis of ABPA provides the opportunity to examine the genetics of a destructive allergic lung disease to specific allergens.

The diagnosis of ABPA is based on a set of criteria outlined in Table 1 (33-35). The criteria consist of clinical and immunologic reactivity to Af. ABPA occurs only in atopic asthmatic and CF patients. The second criteria is that ABPA patients have an elevated total serum IgE concentration >1000 IU/ml. Two of the criteria consist of immunologic reactivity to Af. Patients with ABPA develop IgE and IgG antibody to Af. Initially this was determined by allergy prick skin test and precipitating antibodies. Subsequently, *in vitro* studies demonstrated that the IgE, IgG and IgA anti-Af antibodies were greater than in atopic individual who did not have ABPA (36-40). Immunoblot studies demonstrated that ABPA patients developed IgE, IgA and IgG antibodies to more Af allergens and at a higher concentration than non-ABPA patients (41-43). Recently with the development of studying serologic reactivity to recombinant purified Af allergens difference between ABPA and atopic individual has been observed. Atopic individuals develop IgE antibodies to fewer purified allergens, namely Asp f1 and f3; whereas ABPA patients develop IgE antibodies to more Af allergens, namely Asp f1, f2, f3, f4, f6, f12 and f16 and at greater level (Figure 1). Finally, repeated pulmonary allergic inflammation in response to Af colonization may lead to central bronchiectasis and/or fibrosis. The

Table 1. Diagnostic criteria of allergic bronchopulmonary aspergillosis

- Underlying asthma or cystic fibrosis
- IgE antibodies to *A. fumigatus*
  - positive prick skin test to *A. fumigatus*
  - elevated IgE anti-*A. fumigatus* antibodies
- IgG antibodies to *A. fumigatus*
  - positive precipitating antibody to *A. fumigatus*
  - elevated IgG anti-*A. fumigatus* antibodies
- Eosinophilia, blood and pulmonary
- Elevated total serum IgE >1000 IU/ml
- Central bronchiectasis and/or fibrosis

differences between ABPA and atopic individuals' responses to Af appear to be quantitative and qualitative. In this regard, the differences of ABPA from allergic asthma may provide insight into severe atopic asthma that leads to airway remodeling.

3. HISTOPATHOLOGY OF ABPA

In the pathogenesis of ABPA as seen in Figure 2, Af spores 3-5 microns in size are inhaled and germinate deep within the bronchi into hyphae (44,45). In addition, fragments of the hyphae can be identified within the interstitia of the pulmonary parenchyma as well. The implication of this is that there is the potential for high concentrations of Af allergens exposed to the respiratory epithelium and immune system. *A. fumigatus* releases a variety of proteins, including superoxide dismutases, catalases, proteases, ribotoxin, phospholipases, hemolysin, gliotoxin, phthioic acid and other toxins (46-64). The first line of defense against Af colonization in the lungs is macrophage and neutrophil killing of the condidia and the hyphae (65-68). This is evident in patients with defective phagocytic killing, such as chronic granulomatous disease who are susceptible to invasive pulmonary aspergillosis. In the development of ABPA, Kauffman's group proposed that Af proteins have a direct effect on the pulmonary epithelia and macrophage inflammation. They demonstrated that Af proteases induce epithelial cell detachment (69-73). In addition, protease containing culture filtrates of Af induce human bronchial cell lines to produce pro-inflammatory chemokines and cytokines, such as IL-8, IL-6 and monocyte chemoattractant protein (MCP)-1. In addition, we have observed that Asp f2/, f3 and f4 stimulated cultures stimulate MNC IL-10 synthesis. Thus, various Af proteins have significant biological activity that disrupts the epithelial integrity and induces a monokine inflammatory response (73, 74). Other fungi, such as *Alternaria* and *Cladosporium* and Der p1 from house dust mite also produce proteases with similar biologic activity, but Af proteases are more potent (73, 75). This protease activity allows for enhanced allergen exposure to bronchoalveolar lymphoid tissue (BALT) immune system.

4. BRONCHOALVEOLAR LYMPHOID TISSUE (BALT) IN ABPA

There are several lines of evidence that the BALT initiates and maintains an immune response to Af with subsequent trafficking to the peripheral lymphoid system (76-79). Greenberger and colleagues demonstrated that IgE and IgA anti-Af antibodies were primarily made in the BALT; whereas IgG anti-Af antibodies were produced in the peripheral lymphoid tissue (80). Furthermore, specific IgE anti-Af antibodies represented only a fraction of the total elevated IgE levels and that the majority of the total IgE was synthesized by the peripheral lymphoid system. In lung biopsies of CF ABPA patients, lymphoid follicles containing predominantly IgE+ B-cells were observed, supporting BALT IgE synthesis (81). Analysis of cells obtained from bronchoalveolar lavage fluid (BALF) in ABPA reveal that they are an admixture of alveolar macrophages, eosinophils and lymphocytes, similar to that found in asthma. Eosinophil infiltration predominates both in BALF and lung tissue as is evident in lung biopsy (44). In addition, eosinophils are activated and have released their mediators, such as major basic protein. Thus, the Th2 cytokine IL-5, which is essential for the activation of eosinophils, is important in the development of ABPA. However, in murine models of ABPA, eosinophils do not appear to be critical to mediate tissue damage, as IL-5 deficient animals or animals treated with anti-IL-5 antibodies still develop the full spectrum of microscopic lesions (82, 83).

In addition to eosinophils, T-, B- and NK-cells are also found in BALF (76-79). The T-cells are an admixture of CD4+ and CD8+ T-cells in an approximate 2:1 ratio. Both CD23+ NK cells and CD23+ CD4+ T-cells obtained from BALF of ABPA patients have been observed, indication of *in vivo* IL-4 stimulation (1). In previous studies of Af stimulated T-cell lines of ABPA, low percentages of CD23+ CD4+ T-cells were observed (84). Recently, we have observed that *in vitro* IL-4 stimulated MNC from CF patients were induced to express 5-10% CD23+ CD4+ T-cells with high density CD23 expression. This was prompted by the observation that in CF ABPA patients there was increased *in vivo* CD23+ CD4+ T-cells. The role of these cells may be T-cell CD23 and B-cell CD21 ligand-counterligand interaction, which augments IgE synthesis (85-92). Similarly, CD23+ NK cells may be an important source of sCD23 and/or NK-cell CD23:B-cell CD21 interactions increasing immunoblast IgE synthesis.

5. LYMPHOCYTES

5.1. Th2 CD4+ T-cells

From human and murine models, Th2 CD4+ T-cells and their cytokines are central to the development of ABPA, as outlined in Table 2 (84, 93-95). The Th2 cytokine IL-4 plays a central role in the allergic inflammatory responses observed in ABPA (85, 86, 96). IL-4 up-regulates cellular activity via binding to the IL-4 alpha receptor (IL-4Rα) (85, 86, 97-100). The IL-4 receptor is a heterodimer comprised of IL-4Rα and the common gamma chair receptor (Cγ), IL-4Rα/Cγ. In

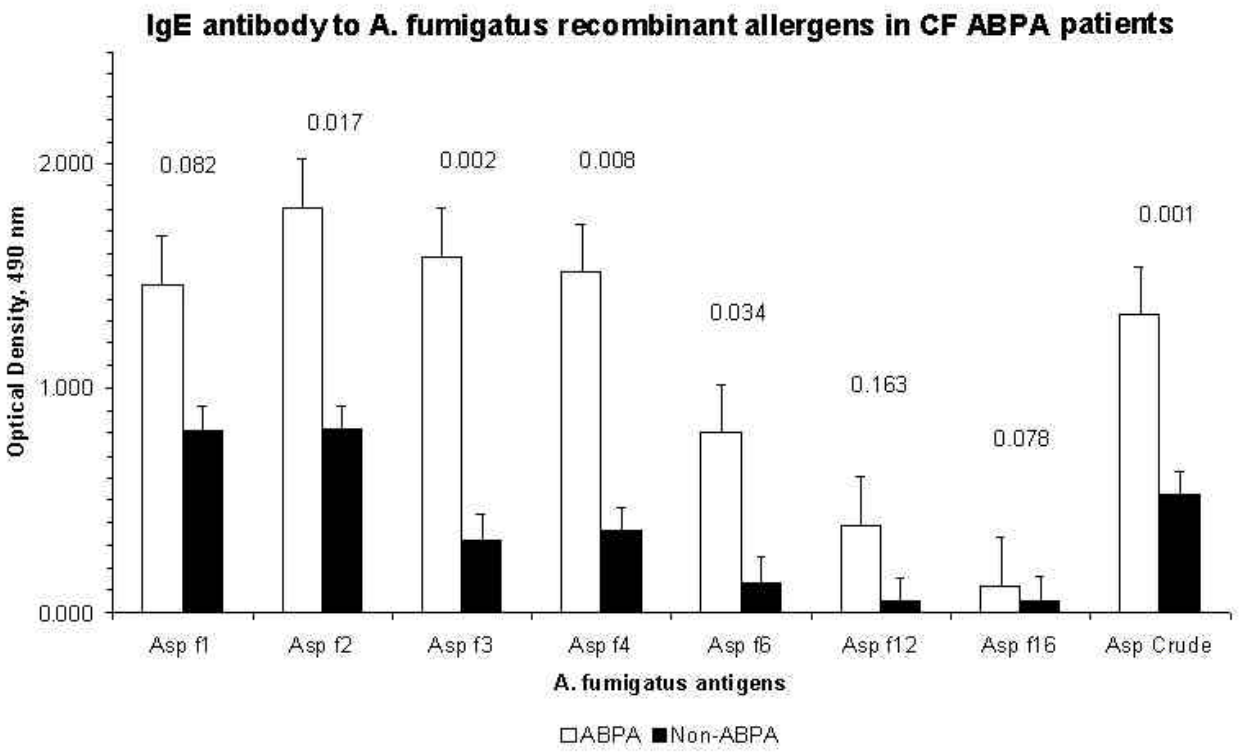

Figure 1. IgE antibodies to purified recombinant *A. fumigatus* allergens in ABPA CF patients. ABPA CF patients had significantly increased IgE antibodies to Asp f2, f3, f4, and f6 allergens. P values above each *Aspergillus* allergen preparation.

Table 2. Th1 and Th2 T-cell cytokines

Cytokine	Sources	Primary Targets	Effects
IL-4	Th2 CD^{4+} T cell	B cell	Growth and activation
	Mast cell		IgE isotype switch
			↑HLA-DR II expression
			↑CD23
		Th1 cell	↓differentiation from Th0 and IFN-γ synthesis
		Th2 cell	↑ differentiation
		NK cell	↓ proliferation
		Endothelium	↑ VCAM expression
		Fibroblast	↑ proliferation
IL-5	Th2 CD^{4+} T cell	Eosinophil	↑ proliferation, chemoattraction, adhesion, activation
	CD^{8+} T cell	Basophil	Primes for histamine release
		B cell	↑ IgE, IgA synthesis
IL-13	Th2 CD^{4+} T cell	B cell	Similar as IL-4
	Mast cell	Monocyte	↑ HLA-DR II expression
		Endothelium	↑ VCAM expression
IL-10	Th0 CD^{4+} T cell	Monocyte	↑ differentiation
	Monocyte		↓ HLA-DR II expression
			↓ IFN-γ and TNF synthesis
			Th2 skewing
		Immature T cell	↑ response to IL-2 and IL-4
			↑ Th2 differentiation
		Th1 T cell	↓ activation and cytokine synthesis
		B cell	↓ response to IL-4 and IgE synthesis
		Mast cell	Co-stimulator
		NK cell	↓ cytokine synthesis
IFN-γ	Th1 CD^{4+} T cell	Monocyte	↑ differentiation
			↑ HLA-DR I and II expression
		CD4+ T cell	Shift of Th2 to Th1 cell
		CD8+ T cell	↑ cytotoxicity
		NK cell	↑ activation
		Endothelium	↑ HLA-DR II expression
			↑ ICAM-1 expression
		Epithelium	↑ RANTES
IL-2	CD4+ T cell	T cell	↑ growth of Ag-specific cells
	NK cell		↑ cytokine synthesis
			↑ maturation of CD8+ T cell
		B cell	↑ growth and Ig synthesis
			IgG1 switch
		NK cell	↑ growth, activation, IFN-γ synthesis
		Monocyte	↑ proliferation, differentiation, IL-1 and TNF synthesis
TGF-β	CD^{4+} T cell	T cell	↓ IL-2 stimulated growth
	Platelets	B cell	counteracts Il-4
		NK cell	↓ cytotoxicity
		Fibroblasts	↓ at low concentrations, ? at high concentrations

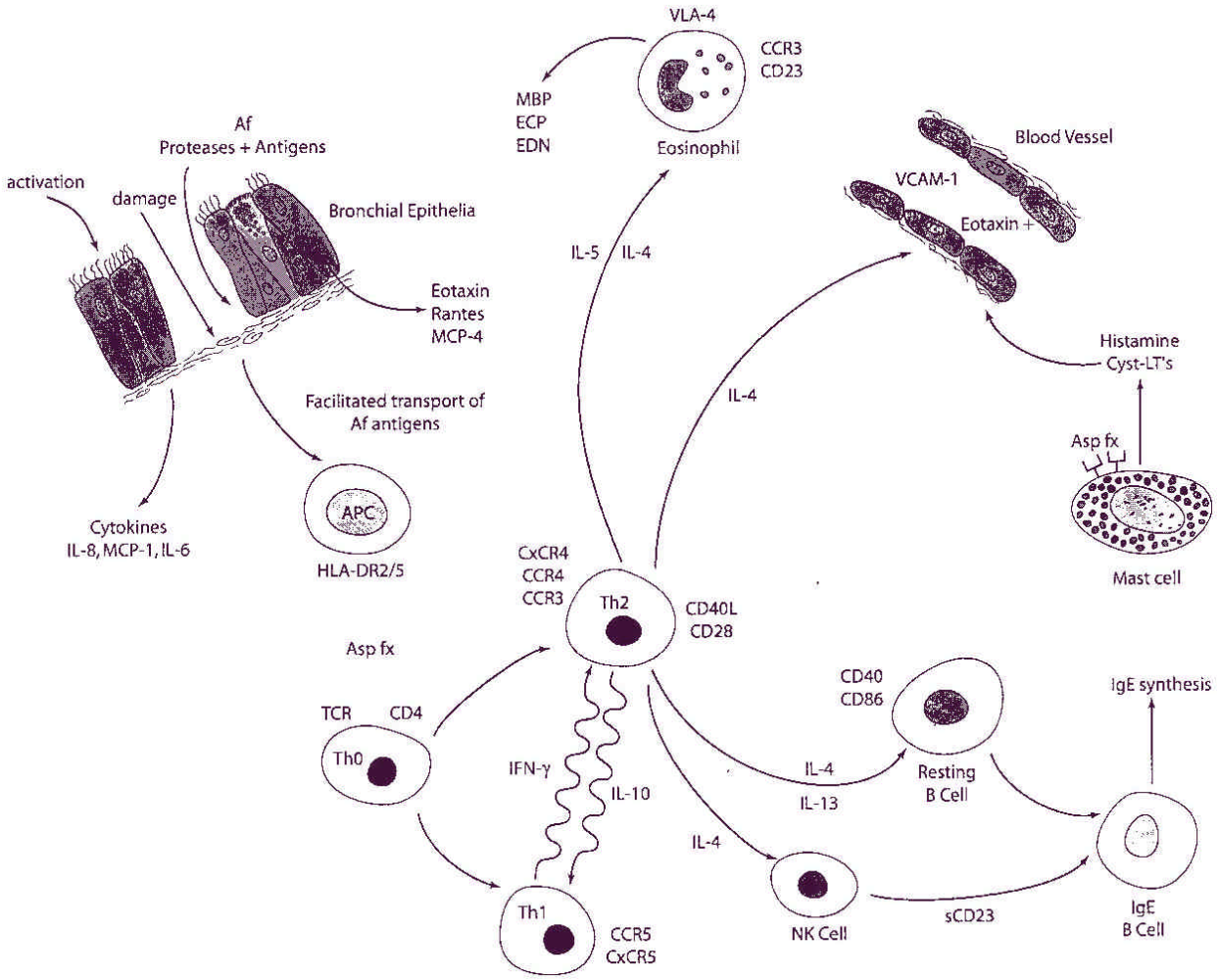

Figure 2. Model of pathogenesis of allergic bronchopulmonary aspergillosis.

addition, IL-4Rα forms a heterodimer with IL-13 alpha (IL-13Rα), IL-4Rα/IL-13Rα. Both IL-4 and IL-13 binds the IL-13 receptor. The IL-4 receptor is present on a variety of cells including B-cells, NK-cells, mast cells, endothelial cells and subpopulation of T-cells. Both IL-4 and IL-13 induce IgE isotype switch of B-cells to IgE synthesis. IL-4 also induces CD23+ expression, the low affinity IgE receptor, and soluble CD23 (sCD23) which augments B-cell IgE synthesis. In Asp f1 T-cell lines, the phenotypes were CD4+CD25+ T-cells and had the cytokine profiles IL-4+ and IFNγ-, indicating Th2 CD4+ T-cells (1, 9). Furthermore, lymphoproliferative responses for Asp f1 T-cell lines were inhibited by anti-IL-4 but not by anti-IL-2, suggesting an IL-4 autocrine response. Interestingly, atopic CF patients without ABPA also developed Asp f1 generated Th2 CD4+ T-cell lines. Chauhan *et al* (101-102) subsequently developed T-cell clones from asthmatic ABPA patients and demonstrated either Th2 (IL-4+, IFN-γ-) or Th0 (IL-4+, IFN-γ+) patterns. Importantly, tetanus toxoid generated T-cell clones showed a Th1 phenotype, indicating that the Th2 response in ABPA is specific to Af allergens and not a generalized Th2 skewing to cell antigens.

In ABPA CF patients, the cytokine pattern to Asp f2, f3 and f4 stimulated 7 days MNC cultures were examined, as seen in Figure 3. ABPA CF patients had significantly increased IL-4 and IL-5 and decreased IFN-γ synthesis compared to non-ABPA and non-atopic controls. Interestingly, IL-10 synthesis was increased in all patient groups to Asp f2, f3 and f4 stimulation, but not to tetanus toxoid stimulation. Moss and colleagues reported decreased IL-10 and normal IFN-γ synthesis following PMA and ionomycin stimulation of tetanus toxoid clones in CF patients (103). However, they did observe normal L-10 synthesis to lipopolysaccharide (LPS) stimulation. Other investigators observed an imbalance of increased pro-inflammatory cytokines, such as TNF-α and IL-8, and decreased anti-inflammatory cytokine IL-10 (104-107). Berger and colleagues showed that cystic fibrosis bronchial epithelial cells have decreased IL-10 synthesis. Thus, IL-10 synthesis in CF ABPA may depend upon the antigen stimulation used. *Aspergillus* appears to stimulate IL-10 synthesis. IL-10 is an anti-inflammatory cytokine synthesized by a variety of cell types, including monocytes, Th1 and Th2 cells, and bronchial epithelial cells. IL-10

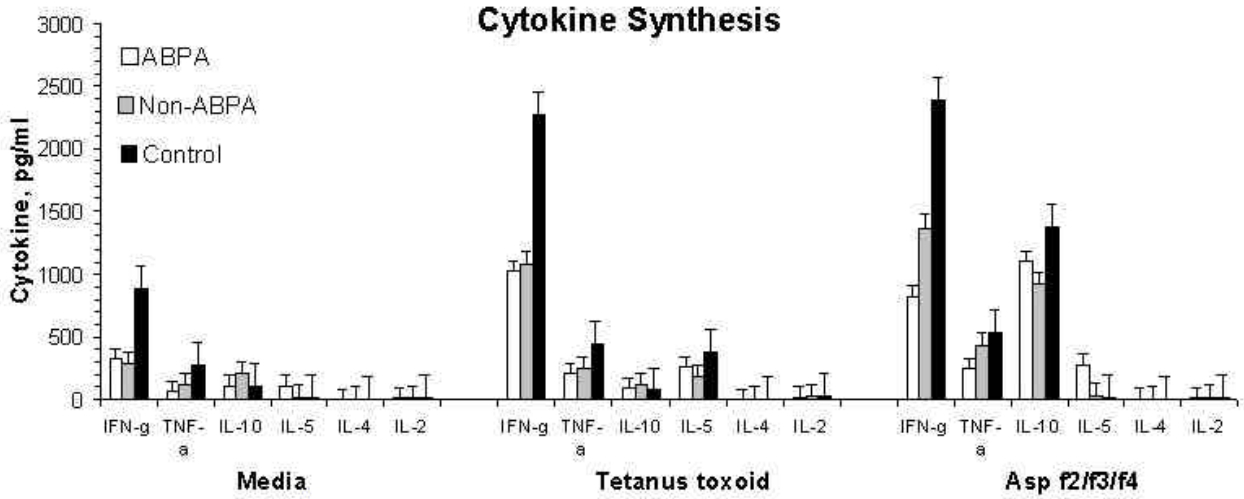

Figure 3. *Cytokine synthesis in ABPA to Aspergillus stimulation.* Following tetanus toxoid and Asp f2/3/4 stimulation, ABPA CF patients had significantly decreased IFN-γ synthesis and increased IL-4 and IL-5 synthesis compared to non-ABPA CF patients and control. IL-10 synthesis was increased in all three groups following Asp f2/3/4 stimulation. Data presented as Mean ± SE.

regulates both Th1 and Th2 lymphocytes though there is a preferential inhibition of Th1 responses (108). In murine models of ABPA, IL-10 inhibited lung inflammation by restricting IL-5 and IFN-γ secretion into the airway lumen (109). The source of IL-10 in these culture studies is unknown but is probably both monocyte and T-cell derived. Af allergens, by stimulating IL-10 synthesis and preferentially inhibiting Th1 response, may promote a Th2 T-cell response toward Af allergens. The frequency of Th1 and Th2 T-cells were also examined in those patients (Figure 4). In ABPA and non-ABPA CF patients, the frequency of PMA and ionomycin stimulated IFN-γ+ CD3+ T-cells was reduced compared to normal controls (110). Though PMA and ionomycin stimulated IL-4+ CD3+ T-cells were not increased in ABPA patients, the frequency Af-stimulated cultures IL-4+ CD3+ T-cells was increased in of ABPA. When tetanus toxoid and Asp f2, f3, and f4 stimulated T-cells were examined, IFN-γ+ CD3+ T-cells were decreased in CF patients. Thus in ABPA CF patients, there is increased frequency of Af-stimulated Th2 T-cells and decreased IFN-γ+ Th1 T-cells. Thus, in CF patients, there is a skewing of Th2 T-cells which probably plays a role in the increased incidence of ABPA in CF patients.

5.2 B-cells

In addition, IL-4 appears to up-regulate CD86 expression in B-cells in atopic patients (85, 86, 97, 111-120). This is important since CD86 in B-cells is an important co-stimulatory molecule for augmentation of IgE synthesis. The ligand for CD86 is CD28 on T-cells. CD86 and CD28 colligation also stimulates T-cells, promoting Th2 CD4+ T-cell responses and cytokine synthesis, eosinophil airway inflammation and airway hyper-responsiveness after allergen challenge. Recently, we have examined the effects of IL-4 stimulation of CD23 and CD86 expression on B-cells of patients with ABPA. In previous studies, purified B-cells from ABPA were observed to spontaneously synthesize elevated amounts of IgE compared to Af sensitive non-ABPA patients, indicating *in vivo* stimulation into IgE secreting immunoblasts (121). In recent studies, CD23 and CD86 expression on freshly isolated B-cells of ABPA patients were increased compared to non-ABPA Af sensitive patients suggesting *in vivo* IL-4 or IL-13 stimulation. Following IL-4 stimulation, ABPA patients had significantly increased rates of CD23 expression per B-cells compared to atopic and non-atopic (Figure 5). IL-4 stimulation did not increase the percentage of CD86 in ABPA patients, but did significantly increase the number of CD23 molecules per CD86+ B-cell. Though IL-13 also increased CD23 expression, there was no significant increase compared to other groups (Figure 6). Thus, ABPA patients had increased sensitivity to IL-4 stimulation with up-regulation of CD23 and CD86 expression compared to other atopic individuals, such that ABPA >atopic >>non- topic patients. The consequences of repeated allergic inflammation in ABPA are central bronchiectasis and/or fibrosis. The inflammatory cells and cytokines involved in this process are unknown. From other studies in asthma IL-13 and/or TGF-β has been proposed.

6. IL-4Rα RECEPTOR

As a potential mechanism for this observation, mutations of IL-4Rα polymorphisms are being evaluated. Polymorphisms of IL-4Rα have been identified in atopic individuals with elevated IgE levels (122-123). Some of these polymorphisms have been associated with a gain-in-function of IL-4 and IL-4Rα interactions promoting increased CD23+ expression and IgE synthesis. Subsequently, seven polymorphisms have been identified that result in increased IL-4 activity. In preliminary studies, homozygous mutations of the Q576R polymorphisms were observed in 2 ABPA patients (124). However, increased

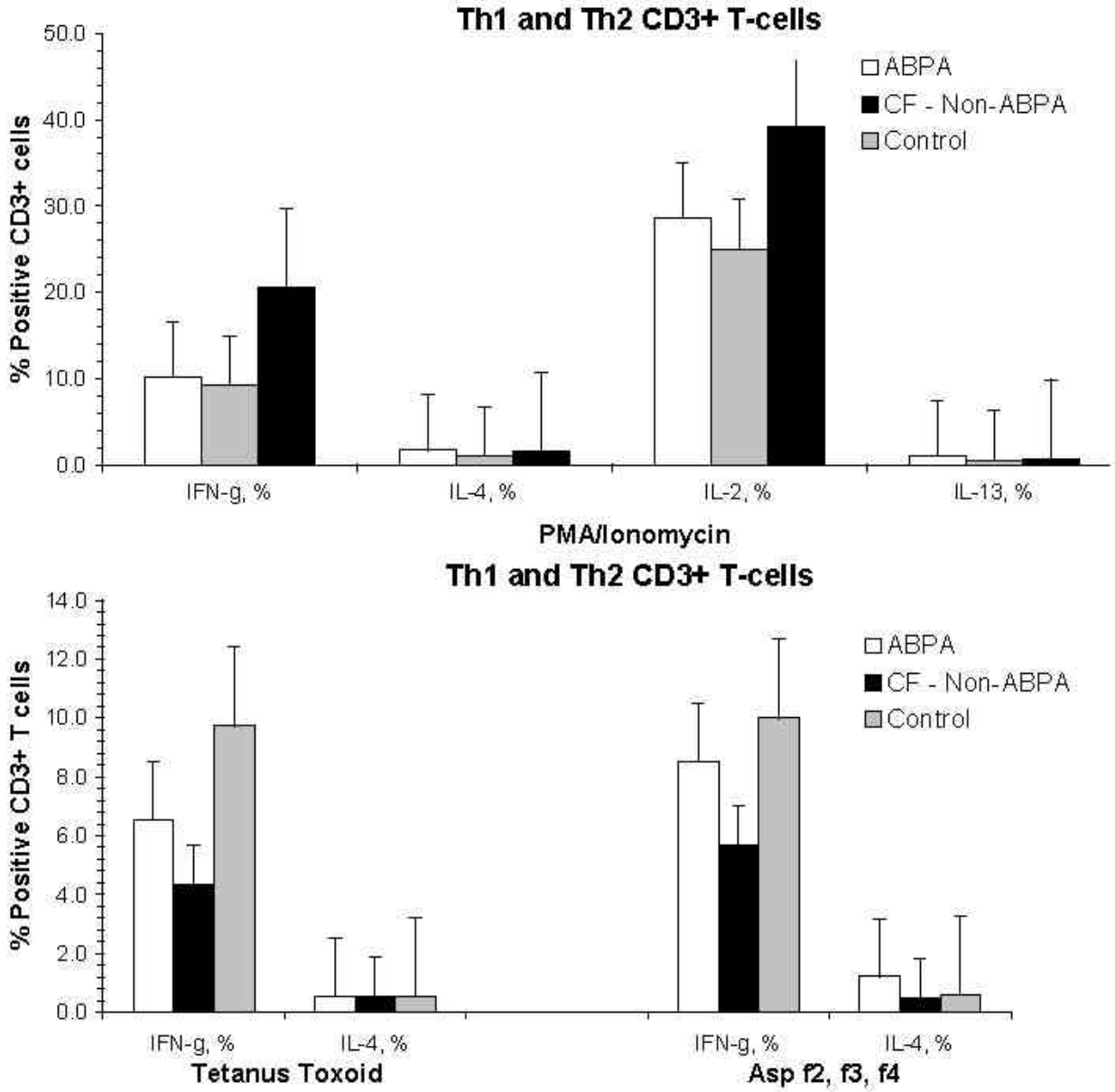

Figure 4. *Th1 and Th2 CD3+ T cells*. Following PMA and ionomycin stimulation (A), ABPA CF patients had comparable percentages of IFN-γ and IL-4 CD3+ T cells compared to non-ABPA CF patients. However, both ABPA and non-ABPA CF patients had significantly decreased percentages of IFN-γ CD3+ T cells compared to normal controls ($p<0.001$, <0.001 respectively). Following tetanus toxoid and Asp f2/3/4 stimulation (B), both ABPA and non-ABPA CF patients had significantly decreased IFN-γ CD3+ T cells compared to normal control ($p<0.05$, <0.05, respectively) but not different from each other. However, ABPA CF patients had significantly increased Asp f2/3/4/ stimulated IL-4 CD3+ T cells compared to non-ABPA CF patients and normal controls ($p<0.01$, <0.01, respectively). Data presented as Mean ± SE. *From* Knutsen AP (106).

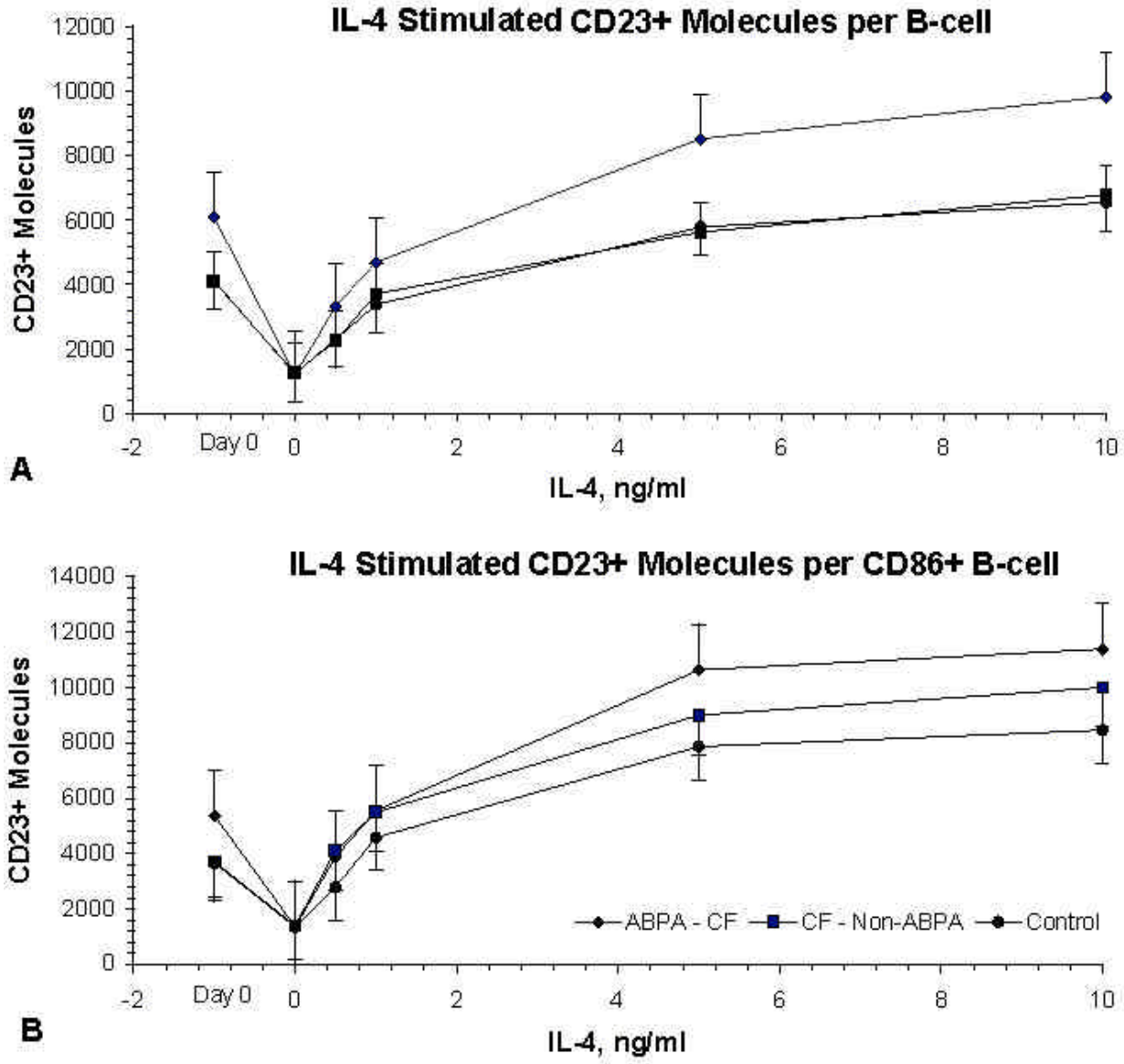

Figure 5. *IL-4 induction* of *CD23+ molecules on CD20+ and on CD86+CD20+ B-cells.* ABPA CF patients had significantly increased number of CD23 molecules per B-cell A and per CD86+ B-cell 3B on day 0 compared to non-ABPA CF patients ($p<0.01$) and non-atopic control patients ($p<0.01$). Following IL-4 stimulation, ABPA CF patients had a significantly increased rate of CD23+ expression per B-cell (A) and per CD86+ B-cell (B) compared to non-ABPA CF ($p<0.01$) and non-atopic control patients ($p<0.01$). Data presented as Mean ± SE. *From* Knutsen AP (106).

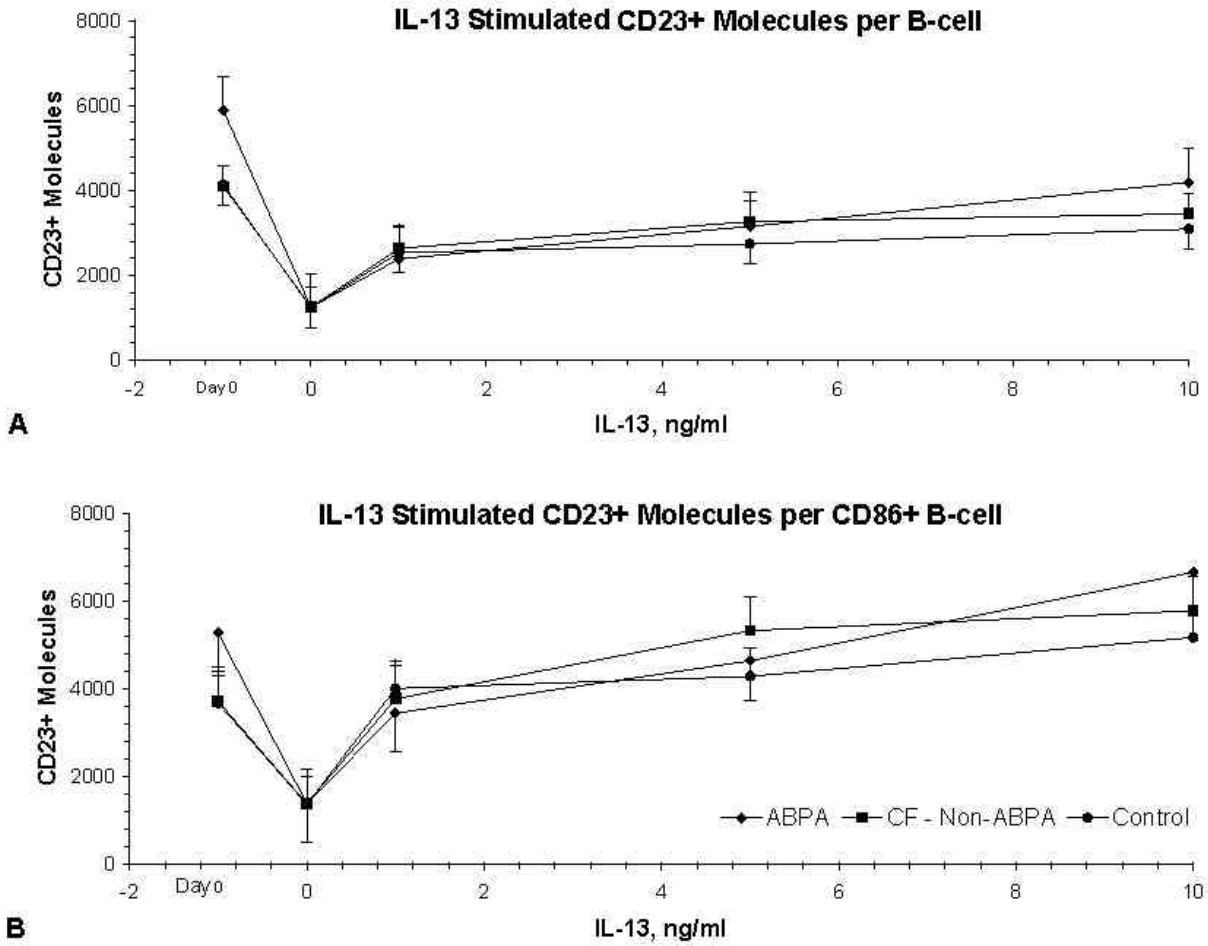

Figure 6. *IL-13 induction* of *CD23+ molecules on CD20+ and on CD86+CD20+ B-cells.* Following IL-13 stimulation ABPA CF patients had no significantly increased rate of CD23+ expression per B-cell (A) and per CD86+ B-cell (B) compared to non-ABPA CF and non-atopic control patients ($p<0.01$). Data presented as Mean ± SE. *From* Knutsen AP (106).

sensitivity to IL-4 stimulation was observed in ABPA and atopic patients without the Q576R polymorphism. Thus, current studies were being conducted to identify all the known polymorphisms in these patients.

7. TCR Vb AND HLA-DR RESTRICTION

Chauhan *et al* (101-102, 125) investigated whether there is unique TCR recognition (T-cell epitopes), TCR-Vβ restriction, or HLA-class II restriction that would promote enhanced Th2 responses. Analysis of T-cell epitope mapping has revealed that there were three immunodominant regions of the Asp f 1 protein in ABPA patients that is recognized by TCR (115). Their findings were similar to that found in other allergen models. O'Hehir and Lamb's group has evaluated T-cell responses to purified house dust mite allergens. In their model, T-cell clones were generated from atopic and non-atopic individuals (116). Significantly, the T-cell clones from non-atopic individuals proliferated in response to allergen stimulation but did not support IgE synthesis, whereas the T-cell clone from atopic patients did. Furthermore, TCR epitope mapping studies revealed limited number of epitopes reacting with TCR (127-128), TCR Vβ restriction or usage (129-130), and HLA class II restriction (130). Four major Vβ chains, Vβ 3,6,13 and 14, react to Asp f 1. This will allow for the evaluation whether mutations of the epitope might alter the T-cell cytokine and/or lymphoproliferative responses for potential immunotherapy of ABPA. Recently, Chauhan *et al* (125) identified that there is HLA-DR2 and DR5 restriction in patients with ABPA. Furthermore, within HLA-DR2 and HLA-DR5, there are restricted genotypes. In particular, HLA-DRB1*1501 and 1503 was reported to provide high relative risk. On the other hand, 40 to 44% of non-ABPA atopic Af-sensitive individuals have the HLA-DR2 and/or DR5 type. Further studies indicated that the presence of HLA-DQ2 (especially DQB1*0201) provided protection from the development of ABPA. These results are similar to those found with purified house dust mite allergens (131-133). Thus, certain genotypes of HLA-DR2 and DR5 may be necessary but not sufficient to cause ABPA. Furthermore, Chauhan *et al* (102) demonstrated that Asp f l allergen has a low-affinity of binding to HLA-DR. This is consistent with Th2 T cell response previously reported by others in that strong antigen HLA-DR-Ag-TCR affinity binding favors a Th1 cellular response whereas low affinity binding favors a Th2 humoral response (132-136).

Table 3. Summary of immunopathogenesis of ABPA

- Th2 CD4+ T-cell response to *Aspergillus fumigatus*
  - BALT mucosal immune response
  - ↑ IL-4, IL-13, IL-5
  - ↓ IFN-γ in CF
  - ↑ IL-10 to *Aspergillus*
  - ↑ IgE total and anti-*Aspergillus* antibodies
  - ↑ IgA & IgG anti-*Aspergillus* antibodies
  - ↑ Eosinophil BALT and peripheral
  - ↑ VLA-4 and VCAM integrins
  - ↑ migration of eosinophils & lymphocytes into inflammatory lesions
  - ↑ Th2 Af-specific responses
- HLA-DR2 (DR15, DR16) and HLA-DR5 restriction
  - genotype restriction
    HLA-DR2: DRB1*1501, 1502, 1503, 1601
    HLA-DR5: DRB1*1101, 1103, 1104, 1202
  - low affinity binding
  - presence of HLA-DQ2 is protective (DQB1*0201)
- TCR-Vβ restriction (Vβ3, β6, β13, β14)
- ↑ IL-4 activity
  - ↑ IL-4Rα polymorphisms
- Mast cell degranulation
  - basophil hyperreactivity
- ↑ sCD25 during ABPA flares
- ↑ T suppressor function
- Anergy to *A. fumigatus* by DTH
- CFTR heterozygous mutations
- Mucus properties (polymorphisms of surfactant SP-A2

8. CHEMOKINES

The role of chemokines, chemokine receptors, and adhesion molecules in ABPA has not been fully investigated (137-143). A number of chemokine receptors have been identified in chemotaxis of eosinophils and Th2 T-cells, namely CCR3, CCR4, CCR8, and CxCR4. Similarly, their ligands are synthesized by a variety of cell types including eosinophils, Th2 T-cells, epithelial and endothelial cells that positively feed back the allergic inflammatory response. The central position that Th2 T-cells and IL-4 plays in ABPA is again seen (144-148). CCL11 (eotaxin) secreted by Th2 T-cells and eosinophils (as well as monocytes, epithelial and endothelial cells) is the most potent chemokine for migration of eosinophils. In addition, eotaxin is expressed on endothelial cells where it interacts with its receptor, CCR3 present on endothelial cells. IL-4 up-regulates both eotaxin synthesis and CCR-3 expression. Other chemokines such as CCL2 (MCP-1), CCL5 (RANTES), CCL7 (MCP-3), CxCL8 (IL-8) and CxCL12 (SD-1α), also induce eosinophil chemotaxis. The principal sources of these chemokines are endothelial cells, macrophages and fibroblasts. The counterligand for eotaxin and RANTES is CCR3, for CxCL12 is CxCR4, for MCP-1 is CCR2, for CxCL8 is CxCR1. The expression of chemokine receptors on T-cells seems to depend upon their differentiation state. Naïve T-cells express CCR7 and CxCR4 with antigen stimulation in the presence of either IL-12 or IL-4, a characteristic chemokine receptor pattern develops. CCR3, CCR4, CCR8 and CxCR4 are induced on Th2 T-cells and the Th1 receptors CCR5 and CxCR3 are down-regulated. Emigration of Th2 T-cells from the blood vessels into the site of allergic inflammation appears to be dependent on eotaxin (CCL11), TARC (CCL17) and MCP-4 (CCL13) from eosinophils and eotaxin from endothelium and smooth muscle. In addition, epithelial cells secrete RANTES (CCL5), eotaxin (CCL11), MCP-4 (CCL13) and MCP-1α (CCL3) promoting both Th2 T-cell and eosinophils into the airway lumen. Thus, IL-4 up-regulates the receptors for these chemokines enhancing migration of eosinophils and Th2 T-cells into the allergic inflammatory site. Similarly, IL-4 also up-regulates expression of the adhesion molecules VLA-4 on T-cells and eosinophils and VCAM in endothelial cells, again promoting selectively of cells involved in the allergic inflammation.

In summary, a quantitative increased Th2 CD4+ T-cell response to *Aspergillus* in both the BALT and systemic immune systems characterize ABPA (Table 3). Perhaps key in the immunopathogenesis is that the BALT is exposed to high levels of Af allergens that have a disrupting effect on the respiratory epithelium allowing for Af allergens causing monocytes pro-inflammatory response. In addition, Af allergens may stimulate high IL-10 synthesis skewing a Th2 T-cell response. Then abnormal mucus properties due to CFTR mutations may promote growth of Af. Other genetic factors are characterized by restricted TCR-Vβ usage and by HLA-DR 2 and DR5 restriction to Af allergens with low affinity antigen binding. This also promotes a Th2 T-cell response. Thus, there is host immunogenetic susceptibility to develop ABPA which resides within the HLA-DR-Ag-TCR signaling of the T-cells and biologic properties of Af that skew a Th2 $CD4^+$ T-cell response.

9. ACKNOWLEDGMENTS

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Send Correspondence to: Alan P. Knutsen, M.D, Pediatric Research Institute, St. Louis University Health Sciences Center, 3662 Park Avenue, St. Louis, Missouri 63110, Tel: 314-268-4014, Fax: 314-577-8459, E-mail: knutsenm@slu.edu