ASSOCATION OF POLYMORPHISMS IN INTERLEUKIN-10 AND MYELOPEROXIDASE WITH INFECTION IN ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

by

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Acute lymphoblastic leukemia (ALL) is the most common malignancy occurring in childhood and accounts for 77% of all leukemia cases. Long-term survival is greater than 80% with appropriate treatment. Chemotherapy is the most widely used treatment, but it can have significant side effects including neutropenia and immunosuppression.

Interleukin (IL-10) is an immunoregulatory cytokine with anti-inflammatory effects. It inhibits some cells like macrophages while stimulating other cells like B cells. IL-10 has been found to be involved in conditions involving an immune response and inflammation, including pneumonia, septic shock, and graft versus host disease (GVHD).

Myeloperoxidase (MPO), which catalyzes the production of hypochlorite from chlorides and hydrogen peroxide, is an abundantly expressed hemoprotein with anti-microbial effects and a major player in host defense. MPO has been implicated in the pathogenesis of a number of conditions and associated with certain infections.

In this study, polymorphisms in MPO and IL-10 were investigated to test their association with risk of infection in a population of Caucasian patients with ALL. Genotyping was performed by enzyme digest for IL-10 polymorphism -592(C/A) and by pyrosequencing for the MPO polymorphisms -129(G/A) and -463(G/A). Allele frequencies at each site were in Hardy Weinberg Equilibrium (HWE). No significant correlation was found between the genotype at IL-10 -592, MPO -129 or MPO -643 and infection episodes in our patient population. As the study population was relatively small, no strong conclusions can be drawn, but implications for further research can be identified.

Public Health Significance: Knowledge of the effects of certain genetic polymorphisms may be important when treating patients with ALL. Patients at increased risk of infection may require prophylaxis or more intense surveillance to ensure a better outcome.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Background on IL-10

Interleukin-10 (IL-10) is an immunoregulatory cytokine produced by a number of cell types. This cytokine plays an important role in suppression of the immune response as well as regulating production of other immune cells including the T cells, B cells, natural killer cells, antigen presenting cells, mast cells, and granulocytes. In fact, it was initially discovered as an inhibitor of T helper 1 cells (Fickenscher et al, 2002; Asadullah et al, 2003). IL-10 was first recognized in 1991 as human cytokine synthesis inhibitory factor (CSIF) as it was found to be produced by mouse T helper 2 cells to inhibit T helper 1 cells. Soon after, IL-10 was found to be homologous to an open reading frame of the Epstein-Barr virus (Vieira et al, 1991; Moore et al, 2001). Since the discovery of some of the functions of IL-10, there have been a number of studies investigating the role of this gene in a number of conditions involving an immune response and inflammation including pneumonia, septic shock, graft versus host disease (GVHD), malignant and autoimmune conditions (Fumeaux and Pugin, 2002; Gallagher et al, 2003; Lin et al, 2003; Mazur et al, 2004). The role of IL-10 in the immune system is much more complex than originally thought (Mosmann, 1994).

Genetics of IL-10

Human IL-10 (IL-10) has been mapped to chromosome locus 1q31-32 and encodes 5 exons encompassing 5.1 kb. This cytokine is a homodimer that has a molecular mass of 37 kDa. Each monomer has a molecular mass equal to half of the total and consists of 160 amino acids. Human IL-10 demonstrates 80% homology to mouse IL-10. In addition, there are a number of viral homologs including Epstein-Barr virus, cytomegalovirus, and herpes virus type 2 (Asadullah et al, 2003). Expression of the gene leads to a 2 kb mRNA (Moore et al, 2001). IL-10 is expressed in response to several factors, including endotoxin, tumor necrosis factor, catecholamines, and cAMP-elevating drugs.

Structure of the IL-10 protein

Only human IL-10 is known to have a three-dimensional structure forming a V-shaped dimer, with each monomer rotated 180º to the other (Asadullah et al, 2003). Each arm of the dimer consists of a total of six amphipathic helices. Four of the helices are derived from one subunit (A-D) while the remaining two are derived from the other subunit (E-F). The C-terminal of helices E and F extends into the N-terminal of the A-D subunit of the other dimer (Fickenscher et al, 2002). Furthermore, helices A, C, D, and F of each domain form a left-handed four-helix bundle (see Figure 1). This formation has been found in all helical cytokines.

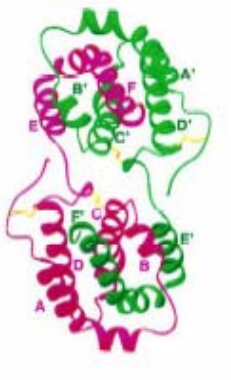

Figure 1: Stereo diagram of IL-10 dimer. Each monomer is a different color (violet and green) and the yellow represents disulfide bonds (Zdanov, 2004)

Disulfide bridges are formed between helices A, C, and D which forms a border with a hollow area in the middle (see Figure 1). Because the internal surface of this border is hydrophobic, amphipathic helices E and F cover the area. 86% of the hydrophobic residues form the hydrophobic core. Subunits are also held together by two flexible polypeptide links that allow for freedom to change the angle between each subunit (Zdanov, 2004).

Function of IL-10

IL-10 has a number of effects with the overall role of regulating the immune response. IL-10 is expressed in T cells, macrophages, monocytes, dendritic cells, mast cells, B cells, eosinophils, keratinocytes, epithelial cells, and a number of tumor cell lines (Mori and Prager, 1998). The main functions of IL-10 include restricting and ending inflammatory response, preventing pro-inflammatory cytokine secretion, and regulating the differentiation and proliferation of immune cells (T cells, B cells, natural killer cells, and mast cells) (Williams et al, 2004). IL-10 is an important cytokine because it is necessary that the immune response be controlled as an

excessive response can lead to damage to the host and can also lead to a number of conditions (Moore et al, 2001; Williams et al, 2004).

Macrophage activation is inhibited by IL-10, leading to reduced expression of pro-inflammatory cytokines including tumour necrosis factor (TNF-α), IL-1, IL-12, IL-6, and granulocyte-macrophage colony-stimulating factor, inflammatory enzymes, chemokines, and several other hematopoietic cells (Williams et al, 2004). Production of IL-10 by macrophages is also inhibited by IL-10 and, therefore, is self-limited. However, it is produced later than the other cytokines and so a considerable amount of these can be secreted before they are inhibited. Blocking the synthesis of TNF-α inhibits the killing activity of macrophages. In addition, IL-10 prevents the expression of MHC class II antigens on several classes of monocytes/macrophages (Mosmann, 1994). Antigen presentation to T cells is blocked as a result of this.

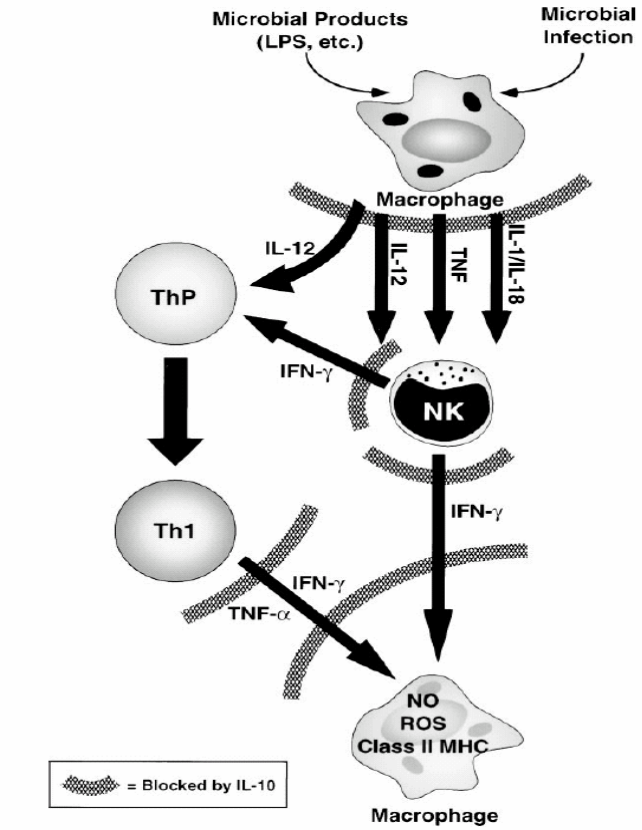

Figure 2: Overall role of IL-10 in immune response to infection. (Akdis and Blaser, 2001)

IL-10 also has some direct effects on T cells (see Figure 2). Suppression of IL-2, interferon-γ (IFN-γ), IL-4, and IL-5 production leads to inhibitory effects on CD4+ T cells (Williams et al, 2004). Proliferation and differentiation of T cells is also indirectly affected by IL-10 when it inhibits IL-2 (Mosmann, 1994; Williams et al, 2004).

IL-10 also has effects on natural killer (NK) cells and mast cells. It inhibits production of IFN-γ by NK cells. In addition, IL-10 increases proliferation of mast cell lines and activates

transcription of two mast cell proteases (Mosmann, 1994). Although we understand the role of IL-10, much more remains to be elucidated about its mechanisms of action.

At the same time, IL-10 has a stimulatory effect on B cells. It improves B cell survival as it is a cofactor for their proliferation in response to certain stimuli and can affect B cell differentiation. It also can result in the development of plasma cells from anti-CD40-activated B cells (Mosmann, 1994; Williams et al, 2004).

Polymorphisms of the IL-10 promoter

The promoter region of *IL-10* is highly polymorphic with two (CA)n microsatellites, located at 1.1 (IL-10G) and 4.0 (IL-10R) kb 5' of the transcription site, and 11 single nucleotide polymorphisms. Three of these SNPs, -1082(G/A), -819(C/T), and -592(A/C) are seen relatively often. (Eskdale et al, 1998; Kube et al, 2001; Moore et al, 2001; Asadullah et al, 2003; Schippers et al, 2005). Approximately 75% of the variation that is seen in IL-10 production appears to be genetically determined based on studies of first degree relative and monozygotic twins with meningococcal disease (Westendorp et al, 1997). This variation is most likely transcriptionally regulated which makes the promoter region an important region of the gene to study (Schippers et al, 2005). Preliminary experiments have shown that IL-10 secretion differences in response to lipopolysaccharide(LPS) are due to differences in mRNA production, rather than mRNA stability. This also suggests that the polymorphisms of the promoter may be responsible for this difference (Eskdale et al, 1998). Furthermore, it has been shown that allelic and haplotypic frequencies in *IL-10* vary between ethnic groups, as we would expect (Wang et al, 2004).

Secretion levels have been associated with certain polymorphisms and haplotypes in the promoter region. Although there have been conflicting reports regarding production, the three SNPs, -1082(G/A), -819(C/T), and -592(A/C), form three conserved haplotypes producing high, intermediate, and low (GCC, ATA, ACC respectively) levels of IL-10 (Dickinson et al, 2004). Eskdale, et al. induced IL-10 production with bacterial lipopolysaccharide to study the effects at microsatellites IL-10G and IL-10R independently and as haplotypes. Any haplotype with 3 repeats at IL-10R was associated with lower production of IL-10. The highest production was observed with the haplotype IL10.R2/IL10.G14 (Eskdale et al, 1998).

IL-10 promoter polymorphisms have been found to be associated with GVHD. Lin, et al. confirmed previous findings that polymorphisms located in the promoter region have an effect on hematopoietic stem cell transplantation. Although the exact mechanism is not known, the A allele at -592 was found to be protective, as patients with this allele had a lower risk of severe acute GVHD and death (Lin et al, 2003). Dickinson, et al. found that the low producer haplotype (ACC) in the recipient was associated with severe acute GVHD grades III-IV in HLA-matched sibling haematopoietic stem cell transplant patients treated with cyclosporin or cyclosporin plus MTX. The ATA haplotype (intermediate production) is involved in survival and severe GVHD (2004). In addition, longer microsatellites at position -1064 in recipients of HLA-matched sibling bone marrow transplants were found to be associated with development of more severe acute GVHD. 22.3% of recipients with 12 to 15 repeats developed grade III to IV GVHD versus 3.92% in recipients with smaller repeats. In this study, it was also found that longer repeats associate preferentially with the A allele at position -1082 (Cavet et al, 1999). Keen, et al. did not find that individual polymorphisms affected transplant-related mortality (TRM) in unrelated

donor stem cell transplantation. However, the presence of the R2-G-C-C haplotype in the donor was associated with higher incidence of TRM while the R3-G-C-C was associated with a reduced risk (Keen et al, 2004).

Polymorphisms have also been associated with risk of certain infections. Gallagher, et al. found that the -1082G allele predicts higher expression levels of IL-10 and was observed more frequently in patients with more severe community acquired pneumonia (Gallagher et al, 2003). The -592 A allele was associated with a greater than 2 fold reduction in the odds of developing human T-cell lymphotropic virus Type I and to play a significant role in genetic susceptibility to this virus (Sabouri et al, 2004). IL-10 polymorphisms may also play a role in acquisition and progression of HIV infection (Wang et al, 2004).

In addition, levels of IL-10 have been shown to have an important in role in the outcomes of particular infectious conditions. One example is meningococcal meningitis. Patients with this infection have been found to have a poor or fatal prognosis when there are high serum IL-10 levels. Patients with a milder course and better prognosis had lower levels of serum IL-10 (Schippers et al, 2005). However, in a study of Calmette-Guérin bacillus, exces IL-10 did not affect the ani-mycobacterial immune response (Murray et al, 1997).

Background on MPO

Myeloperoxidase (MPO) was originally called verdoperoxidase by Agner in 1941 because of its deep green color (Agner, 1941). In fact, MPO is what is responsible for the green color of pus. It is an abundantly expressed hemoprotein in neutrophils, accounting for about 5% of neutrophil

protein. The main role of neutrophils is phagocytosis of microorganisms and the release of MPO and hydrogen peroxide into the phagosome (Klebanoff, 1999). It is thought to have a major role in host defense through production of hypochlorite (Eiserich et al, 2002).

Genetics of MPO

MPO has been mapped to an 11 kb region of chromosome 17 at locus q12-24. It has 12 exons and 11 introns. Transcription of this gene only occurs during early stages of myeloid differentiation (Hoy et al, 2002; Klebanoff, 2005). The product of translation is an 80 kD protein that is cleaved to release a signal peptide. Then, N-linked glycosylation and deglucosylation leads to the 90 kD inactive apoproMPO. While in the endoplasmic reticulum, the protein interacts with calreticulin and calnexin so that heme insertion can occur. ApoproMPO becomes the active enzyme, proMPO. Then, two proteolytic cleavages lead to the final product, mature MPO (Klebanoff, 1999).

Structure of MPO protein

The final product of MPO biosynthesis is two dimers each consisting of a heavy and a light subunit. Molecular mass is approximately 150 kDa. The heavy subunits are linked together by a disulfide bond. In addition, a carbohydrate and two hemes per molecule are covalently bonded to the heavy subunit. The bond with the hemes consists of two ester linkages and one sulfonium ion linkage (Klebanoff, 1999; Arnhold, 2004).

Function of MPO

The main function of MPO is to defend the host by producing hypochlorite from chlorides and hydrogen peroxide. Hypochlorite is a potent bactericide that is used by neutrophils to destroy bacteria (Kutter et al, 2000). MPO is released from the azurophilic granules of neutrophils when they are stimulated by bacteria (Oberg et al, 1987).

MPO is released into the phagosome after a "respiratory burst" where there is increased oxygen consumption leading to the production of superoxide anion (O_2^-) by NADPH oxidase (see Figure 3). Hydrogen peroxide (H_2O_2), which is formed by dismutation of O_2^-, and halides (Cl^-, I^-, Br^-, or SCN^-), reacts with MPO to produce hypohalous acids (Hoy et al, 2002). The toxicity of H_2O_2 is greatly increased when it reacts with MPO and chloride. Powerful oxidants, HOCl, OCl^-, and Cl_2, are

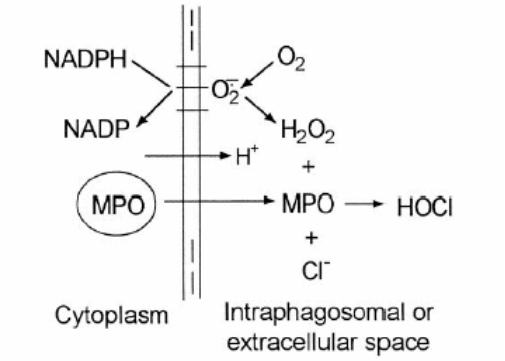

Figure 3: MPO-mediated anitimicrobial system. (Klebanoff, 2005)

responsible for most of the toxic potential of this system, but secondary products also contribute (Klebanoff, 1999). The release of MPO has also been shown to occur during periods of

inflammation. It is a result of this that the association of MPO with non-infectious diseases has become an important area of study (Hoy et al, 2002).

During inflammation, plasma levels of MPO are usually increased and it binds to the endothelial plasma membrane. In addition, tyrosine is nitrated under these conditions to form nitrotyrosine in subendothelial and epithelial tissues. MPO is also involved in blocking the response of neutrophils in a number of ways including inactivation of contents that it secretes (Arnhold, 2004). Overall, MPO protects against microbial infection, but during inflammation, it inadvertently becomes involved in cell damage (Rocha et al, 2002).

Mutations and Polymorhpisms of MPO

A polymorphic site of the promoter, -463(G/A), has been associated with carcinogenesis. The G allele is a strong transcription factor binding site leading to increased levels of MPO expression and has been observed more often in patients with acute promyelocytic leukemia and multiple sclerosis. The A allele is associated with lower levels of expression and has been associated with a decreased risk for lung and other cancers (Reynolds et al, 1997; Klebanoff, 2005). In addition, Rocha et al. found that when bone marrow donor genotype at this locus was AG or AA, there was an increased risk for bacterial infection after bone marrow transplant in HLA-identical siblings, which may be due to the decreased activity of MPO (Rocha et al, 2002).

Levels of MPO are also influenced by another promoter polymorphism, -129(G/A). The A substitution is associated with decreased serum levels of MPO. The A allele eliminates a transcription factor binding site (Hoy et al, 2001).

MPO deficiency, partial or complete, is a relatively common condition affecting 1 in 2000 to 4000 individuals in the United States. This condition can be congenital or acquired. Partial deficiency is characterized by the presence of MPO in half the normal amount. Complete deficiency is characterized by lack of mature MPO protein (Aratani et al, 2000; Hoy et al, 2002; Kameoka et al, 2004). Several mutations have been described in MPO deficiency, making it genetically heterogeneous. C to T substitution at nucleotide 8089 of exon 10 leads to prevention of apoproMPO from becoming mature and active. A missence mutation in the gene leads to a cysteine in place of a tyrosine at position 173 of the amino acid sequence and allows new disulfide bonds to form in the light subunit. This prevents the protein from undergoing proteolytic processing. In addition, a T to C substitution was found to replace a methionine with threonine at amino acid position 251 in Italian patient with complete MPO deficiency (Nauseef, 2004).

MPO deficiency was originally thought to increase risks for severe infections, but some subsequent studies have not proven this except in patients also affected with diabetes mellitus (Hoy et al, 2002). Milla et al. found that MPO deficient mice had increased lung injury after allogeneic marrow transplantation. This is likely a result of the donor T cell inflammatory responses (Milla et al, 2004). A protective effect against myocardial infarction and cardiovascular atheroma has actually been suggested with this condition (Kutter et al, 2000). This may be due to the fact that MPO can also lead to tissue damage by producing reactive oxygen species (Hoy et al, 2003). MPO deficiency has also been implicated in carcinogenesis,

but further studies are necessary in order to clearly understand this condition and its implications (Hoy et al, 2002).

Acute Lymphoblastic Leukemia

In general, leukemias are the most common malignant neoplasms in children. Acute lymphoblastic leukemia (ALL) is the most common, accounting for 77% of childhood leukemia cases. In the United States, the annual incidence is 4.1 per 100,000 children under the age of 15 years (Behrman et al, 2004). ALL involves either B-cells or T-cells at any stage during haematopoiesis. Genetic mutations lead to uncontrollable cellular proliferation, as well as prevention of normal differentiation of cells. This allows for immature blasts to destroy the marrow and compete with healthy tissue (Redaelli et al, 2005).

In almost all cases, the etiology of ALL is unknown, but a number of genetic and environmental factors have been associated with risk for childhood leukemia. Certain genetic conditions predispose children to leukemia including Down syndrome, Fanconi syndrome, and Bloom syndrome (Behrman et al, 2004). However, these account for a rather small proportion of ALL cases. Familial inheritance has also been noted with siblings of affected patients having a 2 to 4 times greater risk of developing ALL. Identification of environmental risk factors, including ionizing radiation while in utero, has been attempted, but evidence is weak (Redaelli et al, 2005). In 2002, a SEER review showed that boys have a slightly greater risk than girls (1.3:1) (McNeil et al, 2002). In addition, a recent literature review found that well developed, industrialized countries had higher rates of ALL (Redaelli et al, 2005).

Long-term survival in children with ALL is greater than 80% after 5 years. The most important prognostic factor is choice of the most appropriate therapy, taking into account stage of the disease, age of the patient, and rate of response to initial therapy. Chemotherapy is the most widely used treatment used for childhood cancers. While chemotherapy destroys malignant cells, it also destroys normal cells that have high rates of turnover including those of the bone marrow and skin. Myelosuppression, including neutropenia and thrombocytopenia and immunosuppression are common adverse effects of this treatment (Behrman et al, 2004). Stem cell or bone marrow transplantation may be necessary in high risk patients including those who relapse or have a poor response to other treatments (Redaelli et al, 2005).

ALL Classification and MPO

The French-American-British (FAB) Cooperative Group has established classification of acute leukemias based on light microscopic detection of myeloperoxidase (MPO) activity in blast cells. This method allows for the determination of the cell lineage of the cancer. MPO levels of less in less than 3% of cells are considered to be diagnostic for acute lymphoblastic leukemia. On the other hand, levels of greater than or equal to 3% of cells is diagnostic of acute myeloid leukemia (AML) (Serrano et al, 1997). In fact, it has been found that ALL responds to treatment with vincristine (VCR) because of the lack of MPO expression. VCR has a complete remission rate of 40% to 60% in ALL patients as opposed to the 10% complete remission rate in patients with AML. Schlaifer, et al. found that the degree of MPO activity is associated with the degree of resistance to VCR. The mechanism by which this occurs has been suggested to be oxidation of VCR mediated by MPO in the presence of hydrogen peroxide (Schlaifer et al, 1993).

Chemotherapy and Risk of Infection

In general, cancer patients have an increased risk of infection as their immune systems are already compromised (Behrman et al, 2004). Chemotherapy further increases this risk as most infections are due to physical barriers, such as the skin and mucosa, being disrupted by this treatment. The use of venous access catheters to administer the chemotherapy also disrupts the host defense system (Oude Nijhuis et al, 2002). In fact, one study showed that use of these catheters is associated with a two to four times increase in risk (Rackoff et al, 1999). Most of these infections result from invasion of microorganisms that are part of the normal host flora of the skin and gastrointestinal tract (Oude Nijhuis et al, 2002).

The most important factor for an increased risk of infection is an absolute neutrophil count (ANC) below 0.5×10^9, or severe neutropenia. The ANC is a measurement of the presence of white blood cells in the blood. The intensity of the chemotherapy regimen plays a role in the duration and intensity of neutropenia. Chemotherapy has also been shown to cause abnormalities of phagocyte function, bactericidal ability, and response of granulocytes to inflammatory stimuli (Oude Nijhuis et al, 2002).

MATERIALS AND METHODS

Study Population

DNA samples were obtained from a pediatric population diagnosed with ALL. These patients received chemotherapy as part of their treatment at the Pediatric Oncology and Bone Marrow Transplant Service at Children's Hospital of Pittsburgh, located in Pittsburgh, Pennsylvania.

Medical History Information

Previously, records from the Children's Hospital of Pittsburgh and Hematology Oncology outpatient clinic were reviewed beginning at the time of diagnosis of ALL through 180 days after the first chemotherapy treatment. The criteria used to define an episode of infection included 1) a single fever equal to or greater than 38.3ºC or two recorded temperatures equal to or greater than 38.0ºC within a 12 hour period OR 2) a positive microbiology or radiology result. Data collection for each episode included documentation of temperatures, symptoms, radiology and microbiology results, white blood cell counts, chemotherapy treatments, medications, and radiation treatment. Then, one researcher summarized the data for each episode of infection.

IL10 –592 Genotype analysis

Polymerase chain reaction (PCR)

A total volume of 50-µL per reaction was used including Mg, buffer, and Taq polymerase(from Invitrogen) and dNTP and primers (see Table 1). Samples were denatured at 95ºC for 5 minutes. Samples were then run through 37 cycles of denaturation at 95ºC for 30 seconds, annealing at 56ºC for 30 seconds, and extension at 72ºC for 30 seconds, then, a final extension at 72ºC for 5 minutes. A subset of samples were analyzed in 1% agarose gel to confirm the presence of DNA.

Table 1: IL-10 primers. Capitalized letters indicate nucleotides that are not present in the IL-10 sequence, but that are used to incorporate a restriction site

IL-10 forward	5'-ggt gag cac tac ctg act ag-3'
IL-10 reverse	5'-act ttc Gag aga ctg gcC tcc tac ag-3'

Mismatched primers, as designed by Tseng, et. al., were utilized. Tseng's study was a multiplex study that required the incorporation or elimination of restriction sites for other enzymes that was not necessary in this study. However, these exact primers were still used (2002).

RFLP analysis

15-µL of PCR product was digested with 5 units of RSA I restriction endonuclease (New England Biolabs) at 37ºC overnight. The reaction was stopped by adding orange G and analyzed by electrophoresis in 3% agarose gel.

Sequencing

Sequencing of 24 laboratory control samples was performed to confirm the accuracy of the RFLP analysis. This was completed by PCR amplification of the selected region (as above), clean-up of amplification product by shrimp alkaline phosphotase reaction, termination with d-Rhodamine, ethanol precipitation, and sequencing by Applied Biosystems 3700 DNA Analyzer. Sequencing data was analyzed using the Sequencher program (version 4.05, Gene Codes Corporation). Genotypes were confirmed with bi-directional sequencing.

MPO GENOTYPE ANALYSIS

Genotyping of the G-463A and G-129A single nucleotide polymorphisms was done by previously described methods (Hoy et al, 2003; Pecoits-Filho et al, 2003).

Table 2: MPO primers.

MPO -129 forward	5'-ggt ata ggc aca caa tgg tga-3'
MPO -129 reverse	5'-cct cca cag ctc acc tga tat-3'
MPO -463 forward	5'-cgg tat agg cac aca atg gtg ag-3'
MPO -463 reverse	5'-gca atg gtt caa gcg att ctt-3'

Polymerase Chain Reaction (PCR)

DNA amplification was performed using primers specific to each site (see Table 2).

Pyrosequencing

Both sites were genotyped using pyrosequencing as described by Pecoits-Filho, et al (2003). The products of both PCR reactions were mixed together for pyrosequencing. The sequencing primer was 5'-cct gac ctc aag tga tcc acc-3'.

Data organization

All data, including genotypes and clinical information, was managed using Microsoft Excel created by Microsoft Corporation, 2002.

Statistical analysis

Allele frequencies were calculated and Hardy Weinberg Equilibrium (HWE) principles were used to determine the expected number of individuals with each genotype. The expected frequencies were then compared to the observed frequencies. The Chi-square test was used to determine if the loci were in HWE.

The Wilcoxon Rank Sum Test (Mann Whitney Test) was used to analyze the data for each infection episode. StatXact version 5.0.3 created by Cytel Software Corporation (2001) was used to perform the Wilcoxon Rank Sum Test. The endpoints used to study risk of infection were total number of infections, number of infections with neutropenia, number of infections without neutropenia, and total number of days with fever. The value of each these endpoints after the start of chemotherapy was also analyzed. Each site was studied independently of the others. Heterozygotes and homozygotes for the polymorphism were grouped together as there was a low incidence of the AA genotype for all sites.

RESULTS

Genotype Data

Of the 105 ALL patients, 88 were successfully genotyped for the MPO-129 polymorphism, 79 for the MPO-463 polymorphism, and 77 for the IL-10 -592 polymorphism. Failure to genotype was either due to lack of DNA or failure to amplify the DNA. These numbers do not include the 4 African American and 1 Asian patient because they were excluded from this study as allele frequencies may be different in these populations. All of the patients analyzed were Caucasian. Table 3 shows a summary of the observed genotypes for the study population.

Table 3: Observed Genotypes Promoter Site Genotypes for ALL Patients

IL-10 -592	# Patients		MPO -129	# Patients		MPO -463	# Patients
CC	43		GG	79		GG	45
AC	28		AG	8		AG	25
AA	6		AA	1		AA	9

Key to Base Pairs:
C = Wild Type
A = Polymorphism

G = Wild Type
A = Polymorphism

G = Wild Type
A = Polymorphism

Allele Frequencies

Allele frequencies were calculated at each locus based on the observed genotypes and HWE principles. Table 4 shows these allele frequencies and their 95% confidence intervals.

Table 4: Allele Frequencies

IL-10 -592		**95% CI**
p	0.740	0.673, 0.806
q	0.260	0.194, 0.327
MPO -129		**95% CI**
p	0.943	0.910, 0.976
q	0.057	0.024, 0.090
MPO -463		
p	0.728	0.661, 0.794
q	0.272	0.206, 0.339

In this study, p represents the wild type allele and q represents the polymorphic allele. The allele frequencies of MPO –463 and IL10 -592 were noted to be similar. The polymorphic allele was rarely observed at MPO -129.

Observed v. Expected Genotypes

Table 5: Observed versus Expected Genotypes

IL-10 -592	Observed	Expected
CC	43	42.2
AC	28	29.6
AA	6	5.2
MPO -129	**Observed**	**Expected**
GG	79	78.2
AG	8	9.5
AA	1	<1
MPO -463		
GG	45	41.9
AG	25	31.3
AA	9	5.8

Table 5 shows the expected and observed number of each genotype based on the allele frequencies from table 4 and standard HWE principles. The expected and observed genotypes were noted to be similar and chi square analysis confirmed that these loci are in HWE.

Infection

Most of the patients had genotypes for all three loci, but there were a few exceptions. Overall, there were 90 patients that had been genotyped at one or more of the loci. The sample population includes 59 males and 31 females. Age at diagnosis of ALL ranged from less than one month to 17.67 years, with an average age at onset of 14.25 years. Information regarding each infection episode was reviewed and included first and last date of fever or infection,

whether it was prior to or after beginning chemotherapy, and ANC. All patients were treated with chemotherapy, but the intensity of treatment varied based on the diagnosis. Only 2 of the patients did not have any fever or infection during the study period. Seventeen patients only had fever or infection prior to beginning treatment with chemotherapy.

The majority of the infections and fevers had either a microbiological cause or clinical manifestations suggesting infection. Eighteen of the total number of infections was attributed either to surgery, drug, or transfusion. However, as this was a relatively small number and an infectious cause could not entirely be ruled out, these episodes were included in the analysis. An episode with neutropenia was defined as an ANC less than $500/mm^3$ and an episode without neutropenia was defined as an ANC greater than or equal to $500/mm^3$. Total number of infections was used to determine if there was a difference in risk for infection. These were then broken down into episodes with neutropenia, episodes without neutropenia, and total days of fever as neutropenia and fever are often associated with infections. The total number of infections after chemotherapy was also analyzed separately from the overall total number of infections to determine if the chemotherapy played a role in the risk.

Statistical Analysis

Statistical analyses were performed on the available data. Chi square tests and the Wilcoxon Rank Sum Test (Mann Whitney Test) were used to analyze this data. The chi square test showed that the population was in HWE at each locus. The Wilcoxon Rank Sum Test was used to see if there was a significant association between the genotype at one locus and counts of infection.

At the IL10 -592 locus, there was not a significant association between genotype and total infections (p=0.9094), total infections with neutropenia (p=0.4459), total infections without neutropenia (p=0.2745) or for total days of fever (p=0.3003). In addition, a significant association was not found between genotype and total infections after chemotherapy (p=0.602), total infections after chemotherapy with neutropenia (p=0.613), total infections after chemotherapy without neutropenia (p=0.5397), or total days of fever after chemotherapy (p=0.6076).

At MPO -129, no significant association was found between the genotype and total infections (p=0.2361), total infections with neutropenia (p=0.3456), total infections without neutropenia (p=0.2948) or for total days of fever (p=0.2948). In addition, a significant association was not found between genotype and total infections after chemotherapy (p=0.2274), total infections after chemotherapy with neutropenia (p=0.1796), total infections after chemotherapy without neutropenia (p=0.8895), or total days of fever after chemotherapy (p=0.2131).

For MPO -463, no significant association was found between the genotype and total infections (p=0.757), total infections with neutropenia (p=0.7979), total infections without neutropenia (p=0.4041) or for total days of fever (p=0.9577). In addition, a significant association was not found between genotype and total infections after chemotherapy (p=0.881), total infections after chemotherapy with neutropenia (p=0.6972), total infections after chemotherapy without neutropenia (p=0.6016), or total days of fever after chemotherapy (p=0.524).

DISCUSSION

In our analysis, we did not detect an association between genotype at either MPO -129, MPO -463 or IL10 -592 and risk of infection in ALL patients. The lack of significance in our result is to a certain extent due to the small sample size. Although there does not appear to be a relationship between genotypes at these three loci and risk of infection in this population, it cannot be entirely ruled out based on this study alone.

Immune and inflammatory reactions are very complex and are controlled by a number of cytokines and other proteins. For this reason, it is difficult to predict which of these proteins, if any, may be a risk factor for infections in patients with ALL. It is, in all probability, a result of a combination of them rather than a single one.

Allele Frequencies

Allele frequencies for IL-10 -592, MPO -129, and MPO -643 were analyzed for pediatric patients with ALL. The allele frequencies at each of these loci were noted to be in agreement with HWE. Expected and observed genotypes were in close agreement and illustrates that the study population is a representative, random sample of the Caucasian population. However, for MPO -129, it was noted that there were very few individuals with either one or two copies of the polymorphic allele.

IL-10 -592

No association was found between the -592A polymorphism and risk of infections in our patient population. Other studies have shown the importance of a haplotype association with IL-10 production as opposed to just one SNP being responsible for it. As discussed previously, the SNPs at -1082, -819, and -592 form three common haplotypes. The GCC haplotype is associated with high production of IL-10, the ATA with intermediate production, and the ACC with low production (Dickinson et al, 2004). Therefore, a C at position -592 is associated with high and low production of IL-10 depending on the nucleotide present at the other two SNPs. Other studies have shown other SNPs and the two microsatellites to be part of the haplotype that may affect IL-10 production (Eskdale et al, 1998; Schippers et al, 2005). Furthermore, a study of twins emphasizes the importance of the SNP at position -1082. It has been suggested that an A in place of a G at this position allows for optimal binding of a transcriptional factor that inhibits the expression of IL-10 (Schippers et al, 2005). Genotyping at these other two loci, especially the one at position -1082, and haplotype analysis should be performed in order to investigate this possible explanation.

Interestingly, Mazur, et al found that levels of IL-10 did not differ in ALL patients from one month to one year after chemotherapy treatment and compared to healthy controls. However, they did find differences in the serum concentrations of three other cytokines, including TNF-α, IL-2, and IL-8 (Mazur et al, 2004). It would be interesting to look at these concentrations during chemotherapy as risk for infection may be related to polymorphisms in these other cytokines and not to levels of IL-10.

IL-10 levels have been associated with the severity to which an infection progresses, but not necessarily with risk of infection. As discussed above, high IL-10 levels are associated with a poor or fatal outcome to meningococcal meningitis. This may be due to this genes anti-inflammatory effects (Eskdale et al, 1999). The polymorphism at position -1082 may also influence the severity of community acquired pneumonia. The G allele is associated with higher IL-10 levels. Patients who survive sepsis and septic shock have higher IL-10 levels that decrease over time, while those who do not survive have levels that remain high over time. (Gallagher et al, 2003). Furthermore, Murray et al suggest that IL-10 may be involved in establishing chronic infections by overriding anti-mycobacterial signals of IFN-γ and maintaining the infections. However, IL-10 levels did not affect the anti-mycobacterial immune response. Studying the type and severity of each infection would provide more insight into this possibility. For example certain infections, such as otitis media, may be less severe than other infections, such as pneumonia.

MPO -129

88 patients with ALL were genotyped for the MPO -129 site with frequency of the G allele being 0.943 and of the A allele 0.057. We did have genotypes on a total of 208 patients with pediatric malignancy, including the 88 ALL patients, among others. Of the 208 patients, only 18 patients had one or two copies of the A allele. This polymorphism has been recently identified with similar genotype frequencies observed. 450 cases and 450 controls were genotyped in a study of MPO polymorphisms and brain infarction. This study also showed that the most frequent genotype in all patients was GG with a frequency of 87.6% in controls and 88.4% in cases. The

frequency of the A allele in this study was 6.8%. A total of 8 out of 900 patients had the AA genotype. This polymorphism was found to be in HWE and in linkage disequilibrium with MPO -643 (Hoy et al, 2003).

In a previous study by Hoy et al, the A allele at -129 was found to be rare with an allele frequency of 0.07, but it was significantly associated with low levels of MPO. However, this only explained 2.6% of the variance. Other factors including smoking, birth control use, and white blood cell count were also found to have an effect on serum concentration of MPO.

MPO -643

A significant difference in risk for infections was not found between individuals with genotype GG versus those with AG or AA at position -643 of the MPO gene. Given the fact that MPO is produced by neutrophils, we would have expected to see a difference in the association between number of infections with neutropenia and number of infections without, but neither was significant. Furthermore, the number of infections with neutropenia did not correlate with the genotype. It may be that the neutropenia masked the effects of this polymorphism. However, if the absolute neutrophil count is low as a result of other factors, including the cancer itself and chemotherapy treatment, then the effect that the genotype has on MPO levels may not be observed.

Myeloperoxidase

Another possible explanation for the results at the myeloperoxidase loci, as mentioned in the introduction, is that, at least in a subset of ALL patients, ALL blast cells usually have less than 3% MPO activity. If levels of MPO are low in most patients with ALL, the genotype at certain polymorphic loci of the *MPO* may have little effect on the overall rates of infection. Future studies should include measurement of serum levels of MPO.

Although we studied numbers of infections, we did not analyze the specific cause of each infection. The evidence for the infection varied and was either based on clinical observation, microbiology, imaging, or serology, with many being unidentified. It is possible that this affected our results. Studies have shown that phagocytes with low levels in MPO have in vitro a mild to moderate problem killing bacteria, but a marked problem killing fungi (Rocha et al, 2002). Aratani, et al. found that the MPO-dependent oxidative system is important for defense of the host against both bacteria and fungi, but that the effect does vary by the species of the pathogen. *C. albicans, C. tropicalis, T. asahii,* and *P. aeruginosa* were not killed in MPO deficient mice, but there was only delayed clearance of *A. fumigatus* and *K. pneumoniae* suggesting that other defense systems can kill these pathogens (Aratani et al, 2000). Furthermore, the consequences of subtotal and total myeloperoxidase deficiency were studied with the conclusion that other mechanisms compensate for the lack of MPO in some cases, depending on the pathogen (Kutter et al, 2000). Unfortunately, the clinical information that we currently have contains the cause of infection and the microbiology for relatively few patients. Although this information may be difficult to obtain, in further studies, this information will be important to analyze.

In addition, individuals with MPO deficiency have not been found to be at an unusually high risk of infection (Eiserich et al, 2002). In fact, more than 95% of patients are asymptomatic. Of those with clinical manifestations, the most common infections are due to certain Candida strains. These Candida infections have been associated with diabetes mellitus. (Rosenzweig and Holland, 2004) It may be that the host can compensate for a lack of MPO through other mechanisms of defense.

Interestingly, Hoy, et al found that MPO serum concentrations were significantly lower in offspring when compared to parents. This is consistent with a previous report that found increased MPO release from polymorphonuclear neutrophils during the aging process (2001). Our study involved a pediatric population that may already have relatively low levels of MPO. There may be other factors, in addition to the patient's genotype, that influence the levels of MPO.

Future Importance

It is important to identify risk factors for infection in patients with ALL who are treated with chemotherapy. These patients have suppressed immune systems as a result of their condition as well as the treatment for it. Certain genes, including *IL-10* and *MPO* have been shown to be involved in risks for certain infections, but their effect in patients with ALL receiving chemotherapy has not been considered. Their role in the host immune system makes them likely candidates for a risk of infection, but this system is a complicated one with many genes playing a role. Classifying high risk patients based on genotypes in these genes would allow for increased

prophylaxis and surveillance of these children and, eventually, may lead to preventative therapies involving these proteins.

Conclusions

Based on this study, genotyping of a total of 88 patients with ALL found that the MPO -129, MPO-643, and IL-10 -592 loci are in HWE. No significant associations were found between the genotypes at these loci and risk for infection among our study population. Although we cannot draw any strong conclusions from this small study, it is probable that further analysis of both of these genes may provide further insight into the mechanisms of infection in these patients. This information would have significant implications for their treatment.

APPENDIX

IRB #0405440: Genetic Determinants of Toxicity and Response to Chemotherapy for Childhood Cancer

University of Pittsburgh

Institutional Review Board

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MEMORANDUM:

TO: Rakesh Goyal, MD

FROM: Christopher Ryan, Ph.D., Vice Chair

DATE: September 20, 2004

SUBJECT: IRB #0405440: Genetic Determinants of Toxicity and Response to Chemotherapy for Childhood Cancer

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Your renewal with modifications of the above-referenced proposal has received expedited review and approval by the Institutional Review Board. **This approval is for analysis of data only.**

Approval Date: **September 17, 2004**

Expiration Date: **September 16, 2005**

The protocol and consent forms, along with a brief progress report must be resubmitted at least **one month prior** to the expiration date noted above for annual renewal as required by FWA00006790 (University of Pittsburgh), FWA00006735 (University of Pittsburgh Medical Center), FWA00000600 (Children's Hospital of Pittsburgh).

Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.

CR:dj

BIBLIOGRAPHY

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Agner K (1941) Verdoperoxidase. Acta Chem Scand 2:1-62

Akdis CA, Blaser K (2001) Mechanisms of interleukin-10-mediated immune suppression. Immunology 103:131-136

Aratani Y, Kura F, Watanabe H, Akagawa H, Takano Y, Suzuki K, Maeda N, Koyama H (2000) Differential host susceptibility to pulmonary infections with bacteria and fungi in mice deficient in myeloperoxidase. J Infect Dis 182:1276-1279

Arnhold J (2004) Properties, functions, and secretion of human myeloperoxidase. Biochemistry (Mosc) 69:4-9

Asadullah K, Sterry W, Volk HD (2003) Interleukin-10 therapy--review of a new approach. Pharmacol Rev 55:241-269

Behrman RE, Kliegman R, Jenson HB (2004) Nelson textbook of pediatrics. Saunders, Philadelphia, Pa.

Cavet J, Middleton PG, Segall M, Noreen H, Davies SM, Dickinson AM (1999) Recipient tumor necrosis factor-alpha and interleukin-10 gene polymorphisms associate with early mortality and acute graft-versus-host disease severity in HLA-matched sibling bone marrow transplants. Blood 94:3941-3946

Dickinson AM, Middleton PG, Rocha V, Gluckman E, Holler E (2004) Genetic polymorphisms predicting the outcome of bone marrow transplants. Br J Haematol 127:479-490

Eiserich JP, Baldus S, Brennan ML, Ma W, Zhang C, Tousson A, Castro L, Lusis AJ, Nauseef WM, White CR, Freeman BA (2002) Myeloperoxidase, a leukocyte-derived vascular NO oxidase. Science 296:2391-2394

Eskdale J, Gallagher G, Verweij CL, Keijsers V, Westendorp RG, Huizinga TW (1998) Interleukin 10 secretion in relation to human IL-10 locus haplotypes. Proc Natl Acad Sci U S A 95:9465-9470

Eskdale J, Keijsers V, Huizinga T, Gallagher G (1999) Microsatellite alleles and single nucleotide polymorphisms (SNP) combine to form four major haplotype families at the human interleukin-10 (IL-10) locus. Genes Immun 1:151-155

Fickenscher H, Hor S, Kupers H, Knappe A, Wittmann S, Sticht H (2002) The interleukin-10 family of cytokines. Trends Immunol 23:89-96

Fumeaux T, Pugin J (2002) Role of interleukin-10 in the intracellular sequestration of human leukocyte antigen-DR in monocytes during septic shock. Am J Respir Crit Care Med 166:1475-1482

Gallagher PM, Lowe G, Fitzgerald T, Bella A, Greene CM, McElvaney NG, O'Neill SJ (2003) Association of IL-10 polymorphism with severity of illness in community acquired pneumonia. Thorax 58:154-156

Hoy A, Leininger-Muller B, Kutter D, Siest G, Visvikis S (2002) Growing significance of myeloperoxidase in non-infectious diseases. Clin Chem Lab Med 40:2-8

Hoy A, Leininger-Muller B, Poirier O, Siest G, Gautier M, Elbaz A, Amarenco P, Visvikis S (2003) Myeloperoxidase polymorphisms in brain infarction. Association with infarct size and functional outcome. Atherosclerosis 167:223-230

Hoy A, Tregouet D, Leininger-Muller B, Poirier O, Maurice M, Sass C, Siest G, Tiret L, Visvikis S (2001) Serum myeloperoxidase concentration in a healthy population: biological variations, familial resemblance and new genetic polymorphisms. Eur J Hum Genet 9:780-786

Kameoka Y, Persad AS, Suzuki K (2004) Genomic variations in myeloperoxidase gene in the Japanese population. Jpn J Infect Dis 57:S12-13

Keen LJ, DeFor TE, Bidwell JL, Davies SM, Bradley BA, Hows JM (2004) Interleukin-10 and tumor necrosis factor alpha region haplotypes predict transplant-related mortality after unrelated donor stem cell transplantation. Blood 103:3599-3602

Klebanoff SJ (1999) Myeloperoxidase. Proc Assoc Am Physicians 111:383-389

Klebanoff SJ (2005) Myeloperoxidase: friend and foe. J Leukoc Biol

Kube D, Rieth H, Eskdale J, Kremsner PG, Gallagher G (2001) Structural characterisation of the distal 5' flanking region of the human interleukin-10 gene. Genes Immun 2:181-190

Kutter D, Devaquet P, Vanderstocken G, Paulus JM, Marchal V, Gothot A (2000) Consequences of total and subtotal myeloperoxidase deficiency: risk or benefit ? Acta Haematol 104:10-15

Lin MT, Storer B, Martin PJ, Tseng LH, Gooley T, Chen PJ, Hansen JA (2003) Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. N Engl J Med 349:2201-2210

Mazur B, Mertas A, Sonta-Jakimczyk D, Szczepanski T, Janik-Moszant A (2004) Concentration of IL-2, IL-6, IL-8, IL-10 and TNF-alpha in children with acute lymphoblastic leukemia after cessation of chemotherapy. Hematol Oncol 22:27-34

McNeil DE, Cote TR, Clegg L, Mauer A (2002) SEER update of incidence and trends in pediatric malignancies: acute lymphoblastic leukemia. Med Pediatr Oncol 39:554-557; discussion 552-553

Milla C, Yang S, Cornfield DN, Brennan ML, Hazen SL, Panoskaltsis-Mortari A, Blazar BR, Haddad IY (2004) Myeloperoxidase deficiency enhances inflammation after allogeneic marrow transplantation. Am J Physiol Lung Cell Mol Physiol 287:L706-714

Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A (2001) Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 19:683-765

Mori N, Prager D (1998) Interleukin-10 gene expression and adult T-cell leukemia. Leuk Lymphoma 29:239-248

Mosmann TR (1994) Properties and functions of interleukin-10. Adv Immunol 56:1-26

Murray PJ, Wang L, Onufryk C, Tepper RI, Young RA (1997) T cell-derived IL-10 antagonizes macrophage function in mycobacterial infection. J Immunol 158:315-321

Nauseef WM (2004) Lessons from MPO deficiency about functionally important structural features. Jpn J Infect Dis 57:S4-5

Oberg G, Simonsson B, Smedmyr B, Totterman TH, Venge P (1987) Myeloid regeneration after bone-marrow transplantation monitored by serum measurements of myeloperoxidase, lysozyme and lactoferrin. Eur J Haematol 38:356-362

Oude Nijhuis CS, Daenen SM, Vellenga E, van der Graaf WT, Gietema JA, Groen HJ, Kamps WA, de Bont ES (2002) Fever and neutropenia in cancer patients: the diagnostic role of cytokines in risk assessment strategies. Crit Rev Oncol Hematol 44:163-174

Pecoits-Filho R, Stenvinkel P, Marchlewska A, Heimburger O, Barany P, Hoff CM, Holmes CJ, Suliman M, Lindholm B, Schalling M, Nordfors L (2003) A functional variant of the myeloperoxidase gene is associated with cardiovascular disease in end-stage renal disease patients. Kidney Int Suppl:S172-176

Rackoff WR, Ge J, Sather HN, Cooper HA, Hutchinson RJ, Lange BJ (1999) Central venous catheter use and the risk of infection in children with acute lymphoblastic leukemia: a report from the Children's Cancer Group. J Pediatr Hematol Oncol 21:260-267

Redaelli A, Laskin BL, Stephens JM, Botteman MF, Pashos CL (2005) A systematic literature review of the clinical and epidemiological burden of acute lymphoblastic leukaemia (ALL). Eur J Cancer Care (Engl) 14:53-62

Reynolds WF, Chang E, Douer D, Ball ED, Kanda V (1997) An allelic association implicates myeloperoxidase in the etiology of acute promyelocytic leukemia. Blood 90:2730-2737

Rocha V, Franco RF, Porcher R, Bittencourt H, Silva WA, Jr., Latouche A, Devergie A, Esperou H, Ribaud P, Socie G, Zago MA, Gluckman E (2002) Host defense and inflammatory

gene polymorphisms are associated with outcomes after HLA-identical sibling bone marrow transplantation. Blood 100:3908-3918

Rosenzweig SD, Holland SM (2004) Myeloperoxidase deficiency and other enzymatic WBC defects causing immunodeficiency. In: Rose BD (ed) UpToDate Online. Vol. 13.1

Sabouri AH, Saito M, Lloyd AL, Vine AM, Witkover AW, Furukawa Y, Izumo S, Arimura K, Marshall SE, Usuku K, Bangham CR, Osame M (2004) Polymorphism in the interleukin-10 promoter affects both provirus load and the risk of human T lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis. J Infect Dis 190:1279-1285

Schippers EF, van 't Veer C, van Voorden S, Martina CA, Huizinga TW, le Cessie S, van Dissel JT (2005) IL-10 and toll-like receptor-4 polymorphisms and the in vivo and ex vivo response to endotoxin. Cytokine 29:215-228

Schlaifer D, Cooper MR, Attal M, Sartor AO, Trepel JB, Laurent G, Myers CE (1993) Myeloperoxidase: an enzyme involved in intrinsic vincristine resistance in human myeloblastic leukemia. Blood 81:482-489

Serrano J, Roman J, Sanchez J, Torres A (1997) Myeloperoxidase gene expression in acute lymphoblastic leukaemia. Br J Haematol 97:841-843

Vieira P, de Waal-Malefyt R, Dang MN, Johnson KE, Kastelein R, Fiorentino DF, deVries JE, Roncarolo MG, Mosmann TR, Moore KW (1991) Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: homology to Epstein-Barr virus open reading frame BCRFI. Proc Natl Acad Sci U S A 88:1172-1176

Wang C, Song W, Lobashevsky E, Wilson CM, Douglas SD, Mytilineos J, Schoenbaum EE, Tang J, Kaslow RA (2004) Cytokine and Chemokine Gene Polymorphisms Among Ethnically Diverse North Americans With HIV-1 Infection. J Acquir Immune Defic Syndr 35:446-454

Westendorp RG, Langermans JA, Huizinga TW, Elouali AH, Verweij CL, Boomsma DI, Vandenbroucke JP (1997) Genetic influence on cytokine production and fatal meningococcal disease. Lancet 349:170-173

Williams LM, Ricchetti G, Sarma U, Smallie T, Foxwell BM (2004) Interleukin-10 suppression of myeloid cell activation--a continuing puzzle. Immunology 113:281-292

Zdanov A (2004) Structural features of the interleukin-10 family of cytokines. Curr Pharm Des 10:3873-3884