Changing concepts of Immunodeficiencies:

Primary Immunodeficiencies in the adult

Presented by

Bharat T Srinivasa

Department of Microbiology and Immunology, McGill University, Montreal

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II Abstract (English)

Primary Immunodeficiencies (PIDs) are defined as inherent defects in the immune system. Over 200 PIDs have been described; many in recent years with improvements in detection and increasing awareness. Multiple paradigm shifts have occurred in our understanding of PIDs. The literature review of this thesis seeks to highlight these shifts with suitable examples. One such paradigm shift occurring is the understanding that these diseases can also affect adults in a broader context. To date, there have been few exclusive analyses of cohorts of adults patients with PIDs. This manuscript-based thesis describes the clinical spectrum of diseases observed in 381 patients referred to the Immune Deficiency Treatment Centre with suspected PIDs. Of these, 196 were diagnosed with PID. This manuscript is in the process of being submitted, and represents Canada's first description of a cohort of patients with adult PIDs, and the largest cohort of exclusively adult PID patients.

Abstract (French)

L'Immunodéficience Primaire (IDP) est définie comme étant un défaut génétique du système immunitaire. Plus de 200 types d'IDPs ont été découverts; entre autre grâce à la prise de conscience de l'incidence de ces maladies et au perfectionnement des techniques de détection. Plusieurs changements de paradigme sont survenus dans le domaine des IDPs. Cette revue de littérature tente à mettre en évidence ces changements de paradigme par le biais d'exemples pertinents. Par exemple, il a été démontré que les adultes peuvent aussi être affectés par ces maladies plus que reconnu précédemment. Jusqu'à ce jour, peu d'analyses ont été effectuées exclusivement sur des cohortes de patients adultes souffrant d'IDPs. Cette thèse décrit le spectre clinique des maladies observées chez 381 patients référés au Centre de Traitement de l'Immunodéficience de l'Hôpital Général de Montréal, et chez qui la présence d'une IDP a été suspectée. Parmi les 381 patients référés, 196 ont été diagnostiqués avec une IDP. Ce manuscrit est en train d' d'être soumis, et représente la première description d'une cohorte de patients adultes souffrant d'IDPs ainsi que la plus large cohorte de ces mêmes patients au Canada.

III Introduction

The study of Primary Immunodeficiencies (PID), a group of disorders characterised by genetic defects of the immune system is a rapidly progressing field. Over 200 immunodeficiencies have been identified, and increasing number of patients are now recognised as having PIDs. Improvements in diagnostic tools and research knowledge have led to improved detection of PIDs, novel genetic aetiologies and corresponding therapeutic interventions. This Masters thesis seeks to highlight some of these advances.

The first portion of this thesis is the literature review. Following a brief background on PIDs, the current paradigm shifts in our understanding of these diseases is highlighted, along with select examples. Given the large number of PIDs currently known, an exhaustive review of each would be impossible. Therefore, the literature review further seeks to classify the current known defects according to defective arm of the immune system, and then describe the most common or most clinically relevant in daily medical practice. Finally, current treatment options for PIDs are described briefly.

The second portion of this thesis is a first author manuscript describing a cohort of adult patients with PIDs referred to the Immune Deficiency Treatment Centre (IDTC) at the Montreal General Hospital. Not much is known about adult patients, and this portion of the thesis seeks to characterise the patients referred to

the IDTC according to their immunodeficiencies. This manuscript has been submitted for review.

Primary Immunodeficiencies provide remarkable insight into the functioning of the human immune system in a natural environment and in our understanding of the evolution of the immune system. The novel PIDs that are being discovered tend to reflect new ways of understanding immune processes and offer a lifeline for patients with these rare defects.

IV Literature Review

1. Background

Primary Immune Deficiencies (PIDs) are often described as being inherent defects in the immune system. Observation and study of the presenting clinical phenotypes and analysis of the underlying genetic aetiologies have revealed novel methods of functioning of the human immune system and thus, Primary Immune Deficiencies have also been referred to as windows into the immune system[1].

The first acknowledged PID to be described was in 1952, by Bruton[2]. Termed Bruton's agammaglobulinemia, the patient studied exhibited a clinical history now known to be characteristic of PIDs: recurrent infections. Since then, over 200 PIDs have been described, with over 100 underlying genetic aetiologies[3]. Heightened interest and advancement of research techniques to study these diseases has led to increasing knowledge of PIDs and their underlying genetic factors, and thus revealed a new method for studying the host immune system. Understanding of the pathogenesis of PIDs has led to increased therapeutic interventions. Treatment options for PIDs include infusion of intravenous immunoglobulins for antibody deficiencies, stem cell therapy for T cell or combined immunodeficiencies such as Wiskott-Aldrich syndrome and congenital defects of neutrophil function and MHC deficiency[4, 5]. Currently, gene

therapy for certain monogenic PIDs such as Severe Combined Immune Deficiency (X-linked SCID), adenosine deaminase (ADA)-deficient SCID and chronic granulamotous disease (CGD) are in various phases of clinical trials[6, 7.]

Primary Immune Deficiencies are thought to be rare, and, for the exception of IgA deficiency (IgAD), have an overall prevalence of 1:10,000 live births[8]. The most common PID is IgAD, a mostly asymptomatic disease with a prevalence of up to 1 in 500 among Caucasians, with decreased frequency among certain Asian populations[9]. The clinically most relevant PID, Common Variable Immune Deficiency (CVID) has prevalence between 1:10000-1:20,000[10, 11]. Much higher frequencies are usually observed in populations with high consanguinity rates or among genetically isolated populations[8]. Due to the relative rarity of PIDs, many countries have set up national registries for PIDs in order to ensure a pooled collection of patients in order to perform suitable analyses of data and to conduct molecular studies[12, 13]. These registries bring together patients from multiple centres across countries. Creation of these registries has enabled researchers to obtain samples from patients with rare immune deficiencies, thus enabling research into the PID in itself and develop and test novel therapeutic interventions (reviewed in [13]). The Canadian Immunodeficiency Society manages the Canadian PID database.

It is often acknowledged that microbes have played an integral part in the evolution of the human species[14]. Infections have been a cause of most deaths in

human history. Life expectancies did not exceed 25 years of age until recently and is still in the forties in certain parts of Africa. Life expectancies have not increased due to an evolutionary improvement in immunity. Instead, current increases in life expectancy can be attributed primarily to improvements in the environment: improvements in hygiene that decreases microbial exposure, vaccines that decrease clinical disease and antimicrobial drugs that have resulted in a decrease of fatal outcomes. These modern developments have masked some or most forms of immunodeficiency, and thus it is often argued that human immunodeficiency, rather than human immunocompetence is the rule rather than the exception [14, 15]. Clinical observance of these underlying immunodeficiencies depends on the interplay between the host and the environment: i.e. the genetic make up of the host and exposure to the microbe that the individual is susceptible to[14, 16].

2. The changing paradigm of Primary Immune Deficiencies.

Epidermodysplasia verruciformis (EV) is a rare disease that clinically manifests as persistent warts on the skin. Beginning in childhood, these patients have a high risk of developing skin cancer. First described in 1922[17], the cause for this disease was highly debated for over four decades. In the late 60's a causative virus was associated with the disease[18, 19], and EV was labelled as a viral infection and as an example of virus-induced oncogenesis[20]. In the late 90s, a susceptibility locus was identified in patients with EV[21, 22]. In 2002, mutations in two novel

genes, *EVER1* and *EVER2* were identified which indicated a Mendelian inheritance of these patients with susceptibility towards human papillomavirus infections[23]. *EVER1* and *EVER2* encode for integral membrane proteins, and are expressed in the haematopoietic lineage and in normal skin cells. Mutations in these genes result in a heightened susceptibility to human papillomavirus, with complete penetrance[24]. Because it was first described in 1922, this disease is often described as the first PID, even though the exact nature of the disease was only identified 80 years later[23, 24].

The above example underlies many contemporary issues with PIDs. There is still no consensus definition for Primary Immune Deficiencies[3]. The classic definition of PID refers to patients with infections caused by many organisms[14, 25, 26]. This definition would be applicable to patients with mutations in HLA class I or II genes and patients with Severe Combined Immune Deficiency (SCID), which result in infections by a wide variety of microorganisms[16]. However, patients with mutations in EVER genes, UNC93B1 and TLR3, defects in the complement system and recently identified mutations in apolipoprotein L-1 are although defined as having some form of immunodeficiency. These individuals are susceptible to infections by human papillomaviruses, Herpes Simplex Virus, gram negative Neisseria bacteria species and infections by *Trypanosoma evansi* respectively[24, 27, 28, 29] . This suggests that a person with a pattern of susceptibility to a narrow range pathogens, if not singular pathogens may also be termed immunodeficient. Indeed, it is currently argued that a person with a PID may not

necessarily be susceptible to solely opportunistic and/or respiratory pathogens, but in fact may be susceptible to a certain class of microorganisms depending on the arm of the immune system affected (reviewed in Chapter 3).

Much confusion arises from the term "immunodeficiency" that is used in conjunction with PIDs[3]. It is often assumed that an "immunodeficiency" is defined by a defect in the "immunological" phenotype, i.e. a defect in the haematopoietic system. However these phenotypes are liable to change with the advances in techniques used to study PIDs[30]. Additionally, the use of haematopoietic defects to define immunodeficiency is insufficient, as EV, a defect of the host response to human papillomaviruses arises from mutations in keratinocytes, which plays an important role in the human innate immune system. Thus, a definition that encompasses the entirety of the human immune system, including cells of the haematopoietic system and cells such as keratinocytes that play an important role in the protecting the host from infections is necessary to properly define these highly heterogenous set of diseases.

It is possible to even question whom to term immunodeficient[3]. Epidemiological studies have showed large frequencies of mutations in alleles of Duffy Antigen Receptor for Chemokines (*DARC*) in populations originating from Africa[31]. DARC is necessary for invasion of cells by the malaria parasite *Plasmodium vivax*[32,33], and a single-nucleotide mutation in the promoter of this gene is sufficient for resistance to *P vivax*[34]. Similarly, resistance to HIV-1

infection is associated with recessive mutations in the extracellular domain of CCR5, which is more common in certain Caucasian populations[35, 36]. It could be argued that in certain regions with endemic infections, individuals without mutations in alleles of *DARC* or *CCR5* could be termed as being immunodeficient, as these individuals would show increased susceptibility to P vivax or HIV-1 respectively[3, 14, 30]. Similar cases could be put forth for resistance towards norovirus, which causes gastrointestinal infections, where individuals with mutations in *FUT2* alleles are resistant to infection by the virus[37]. Since these mutations are inherited in a Mendelian manner, and only a small proportion of the global population carry these alterations that result in resistance towards certain infectious agents, it is possible to argue that Primary Immune Deficiencies, as defined by increased susceptibility to a narrow spectrum of pathogens is in fact the norm, rather than the exception[3,15].

Another misconception that exists about Primary Immune Deficiencies is that PIDs are often monogenic. While this is true for some PIDs, many diseases reflect contributions from non-monogenic mutations in multiple genes that together, confer an immunodeficient phenotype in the host. An example of this would be Common Variable Immune Deficiency (CVID). CVID is an umbrella term for a highly heterogenous set of disorders. About 10-15% of all CVID cases are inherited, and mutations in 4 genes have been associated with CVID[38] (reviewed below). Therefore, while the exact underlying cause of CVID has not been identified, given broad spectrum of defects in both humoral and cell mediated

arms that are observed in CVID, it is highly likely that a number of genetic defects together confer the immunodeficient phenotype observed in these individuals[39].

Leprosy presents an interesting case in the context of Primary Immune Deficiencies[3]. Many debates have arisen over the infectious cause and/or genetic basis of leprosy. It was only recently that a genetic basis was identified for this disease. Susceptibility to leprosy shows familial inheritance and twin studies and family cluster linkage analysis have suggested target loci in chromosome regions 6q25-q26, 6p21, 10p13 and 20p12-p12, indicating a polygenic basis to the susceptibility of these individuals to Mycobacterium leprae[40, 41, 42, 43]. Mapping of regions 6q25-q26 and 6p21 have identified specific genes that may play a role in this enhanced susceptibility. Analysis of the 6q25-q26 region in Vietnamese leprosy patients identified markers in the region overlapping the 5' regulatory region shared by *PARK2* and *PACRG*[44]. PARK2 is a ubiquitination E3 ligase involved in the proteasome complex, and PACRG is hypothesised to be involved in the same pathway. PARK2 plays an important role in controlling proteolysis, cellular oxidative stress and in the regulation of innate immune responses[45, 46, 47]. Similarly lymphotoxin alpha (*LTA*) was identified on 6p21, and interaction of LTA with lymphotoxin beta (LTB) is essential for secondary lymphoid organ development and immune response regulation[45]. So far, genes from only two of the four chromosomal regions linked to susceptibility have been identified. It is possible that many more genes in these regions are capable of co-operatively

regulating host susceptibility to Mycobacterium leprae. The identification of mutations in genetic regions that are linked to immune responses, which result in clinical disease by M leprae are reasons why leprosy is considered a Primary Immune Deficiency and an example of a PID with a polygenic basis[3, 45].

One common misconception of PIDs is that these disorders follow Mendelian patterns of inheritance[3]. While most PIDs whose genetic etiology is known are associated with autosomal recessive genetic defects, there are a number of PIDs with autosomal dominant patterns[48]. One example of a PID with autosomal dominant inheritance patterns is the Warts, Hypogammaglobulinemia, Infections and Myelokathexis syndrome (WHIM syndrome)[48, 49]. WHIM is an extremely rare disease, characterised by recurrent infections, neutropenia, B cell lymphopenia and hypogammaglobulinemia. These patients have a higher susceptibility to bacterial infections rather than viral infections. However, there appears to be significantly increased and specific susceptibility to HPV in these patients. These patients have numerous warts on hands, feet and trunk. A large majority of patients diagnosed as having WHIM were found to have mutations in CXCR4, and all these patients have a mutation that results in truncation of the cytoplasmic C-terminus of the CXCR4 receptor due to a premature stop codon[50]. The truncated CXCR4 results in a hyperactive gain-in-function version of the protein, which results in neutropenia[49]. However, the reasons for B cell dysfunction and susceptibility to HPV in this disease are unknown[49].

It is often thought that PIDs are inherited familial disorders. However, increasing numbers of PIDs are being identified with predominantly sporadic genetic etiologies. One such PID is the Hyper-IgE syndrome, which is characterised by recurrent infections and extreme elevations of serum IgE. Other non-immune symptoms involving skeletal and connective tissue abnormalities are common. Two forms of Hyper IgE syndrome exist: upto 70% of Hyper IgE cases contain a dominant STAT3 mutation[51], while the remaining, though not all have been associated with mutatons in dedicator of cytokinesis 8 (DOCK8)[52] and a single person with mutations in TYK2[53]. An extremely rare disease, it occurs predominantly in childhood. Most cases, especially of the more common dominant negative STAT3 form occur sporadically from *de novo* mutations. Children with this disorder survive into adulthood, although life span is shortened, with death often occurring due to gram negative or fungal pneumonias[54].

Finally, PIDs are often thought to occur in childhood. CVID, the clinically most prevalent PID is a disease that occurs predominantly in adults (reviewed below). CVID is the most commonly diagnosed in adults, between the ages of 25-45[55]. The understanding that PIDs are not necessarily pediatric issues, but represent illnesses from all age spectrums reflects yet another changing paradigm of Primary Immune Deficiencies.

In summary, many paradigm shifts have occurred in the fields of Primary Immune Deficiencies[3]. Only a small proportion of these are indicated in this

review, with select examples. The purpose of this portion of the manuscript was to introduce some evolving concepts in the field of PIDs, and highlight some of the complexities involving study of these diseases. The selected examples described above are indicated in Table 1. A complete review of the paradigm shifts in PIDs has been published previously[3, 30].

Table 1: Select examples of Paradigm shifts in Primary Immune Deficiencies.

Paradigms		Examples
Old concepts	New Concepts	
1. Susceptibility to broad range of pathogens (1 gene, many pathogens)	Susceptibility to narrow range of pathogens (1 gene, 1 pathogen)	• Complement disorders and Neiserria • Apolipoprotein and T evansi
2. Haematopoietic	Non Haematopoietic	• EVER mutations in keratinocytes and HPV
3. Rare, affects a minority of the population	Common; "Rule rather than the exception"	• DARC mutations & resistance to P vivax • FUT2 mutations & resistance to norovirus
4. Monogenic (1 gene, many diseases)	Polygenic (Many genes, 1 disease)	• CVID • Leprosy and PARK/PACRG, LTA
5. Mendelian pattern of inheritance	Non-Mendelian pattern of inheritance	• WHIM syndrome.

	(autosomal recessive)	(autosomal dominant)	
6.	Familial	Sporadic mutations	• Hyper IgE syndromes
7.	Childhood	Adulthood	• CVID

Table 1: Shows a select number of paradigm shifts and corresponding examples in PIDs. References indicated in text. This table follows a similar format to that from Casanova et al[3].

3. Classification of Primary Immune Deficiencies.

The most recent update on PIDs and classification of these disorders was published in December 2009, by the International Union of Immunological Societies Expert Committee on Primary Immunodeficiencies. Co-chaired by Notarangelo, Fischer and Geha, the committee meets every two years to update the classification of PIDs[56]. The classification pattern followed in this literature review will be similar to that followed by the committee. Many PIDs are orphan diseases, and therefore under each category, only the most common PIDs or PIDs with the most substantial amount of knowledge known will be reviewed. The following are the headings used in the classification of PIDs in this literature review and the 2009 manuscript.

1) Combined T and B-cell immunodeficiencies.
2) Predominantly antibody mediated disorders.

3) Other Well Defined Syndromes
4) Disease of immune dysregulation
5) Disease of phagocyte number and function
6) Defects in innate immunity.
7) Complement disorders.

3.1 Combined T and B-cell Immunodeficiencies.

Combined Immunodeficiencies (CID) are a more severe and correspondingly rarer form of PIDs. Overall, CIDs account for 15% of all identified PIDs[57]. A heterogenous group of disorders, most diseases that fit the pattern of CID have defects in either development or function of T lymphocytes[58, 59]. In many CID, defective humoral immunity is observed, and this is either due to defects in the B lymphocyte arm of the immune system or due to defects of T cell help[8, 58]. Indeed, these patients are usually referred to with recurrent and severe infections from a broad spectrum of pathogens, reflecting defects in the entire adaptive immune response. In severe forms of CID (Severe Combined Immune Deficiency; SCID), there are decreased to no circulating functional T cells, which result in poor adaptive immune responses[60]. SCID is asymptomatic at birth, and most infants with SCID are only diagnosed upon severe infections[61]. Infections can be from bacterial, viral or fungal pathogens[8, 58].

According to the latest classification of PIDs, SCID can be categorised into two types: 1) SCID with absence of T lymphocytes but presence of B-lymphocytes (T-B+ SCID) and 2) SCID with absence of both T and B-lymphocytes (T-B- SCID)[56]. Each group can be further classified based on presence or absence of Natural Killer (NK) cells. Both forms of SCID (T-B+ and T-B-) have similar clinical features: early onset, recurrent infections and, without timely detection, failure to thrive. Frequency of SCID varies, with ranges between 1:50,000[8] to more than 1:100,000[62] among live births being reported. SCID is more common among male infants and this is primarily due to the prevalence of X-linked SCID[8].

3.1.1 T-B+ SCID

X-linked SCID, with mutations in the IL-2 receptor γ gene (*IL2RG)*[63] accounts for up to 40% of all SCID cases[8]. The gene encodes for the γ chain (γc), which is shared between receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. Due to defective signalling via the IL-7 and IL-15 cytokines, which plays a role in early T cell development and NK cell development respectively[64]. Infants with X-linked SCID show no circulating T and NK cells, but normal levels of non-functional B cells[8, 65]. Infants with this disorder show symptoms of SCID between ages of 3-6 months, after decrease of circulating maternal antibodies[65]. Interestingly, infants with SCID caused due to mutations in Janus Activated Kinase- 3 (JAK3) have identical symptoms to those patients with mutations in the

γc[66], due to the shared signalling pathways between the γc and JAK3[67]. The only difference between these two forms of SCID is that while γc-SCID is X-linked and therefore found only in male infants, JAK3 SCID is autosomal recessive and affects both males and females equally[65]. Similarly, SCID can be caused due to mutations in IL7 receptor (*IL7R*). Individuals with these mutations show defective T cell compartments, but normal levels of B cell and NK cells[68]. Other mutations that cause T-B+ SCID include CD45[69, 70], components of the TCR complex[71, 72] and Coronin-1A[73].

3.1.2 T-B- SCID

Adenosine Deaminase (ADA) SCID, an autosomal recessive inherited form of the disease accounts for 10-15% of all SCID cases[8]. Many phenotypic forms of ADA-SCID are present, with severe disease occurring in children below 6 months of age, delayed onset of disease in children between six months to the first few years, late onset of disease occurring in adults between second to fourth decade of life and partial ADA deficiency[74]. The link between ADA deficiency and SCID was made in the 1970s [75, 76]. Clinically, ADA-SCID is manifest by recurrent infections. Other non-infectious clinical phenotypes occur frequently, including growth failure, absence of lymphoid tissues, and various hepatic and neurological abnormalities[74]. ADA is required for the deamination of adenosine to inoside, and deoxyadenoisine to deoxyinosine[77]. In individuals with SCID, non-functional ADA results in a build up of toxic phosphorylated metabolites of adenosine and

deoxyadenosine, resulting in apoptosis of lymphoid precursors and defects in T and B cell development[8]. Interestingly, deficiency of Purine Nucleoside Phosphorylate (PNP), another enzyme involved in purine metabolism results in a SCID that is indistinguishable from ADA-SCID[78], suggesting the importance of purines and pyramidines in the functioning of the immune system. PNP-SCID accounts for 1-2% of all cases of SCID[8].

Another form of T-B- SCID is caused to defects in genes involved in V(D)J recombination. V(D)J recombination is an essential process involved in generating the diversity of response observed in the adaptive immune system. Additionally, V(D)J recombination acts as a checkpoint for T and B cell development, and therefore individuals with mutations in these genes have a lack of T and B lymphocytes[79], corresponding defects in the adaptive immune system and heightened susceptibility to a broad spectrum of pathogens. Defects in V(D)J recombination can be split further into two types: 1) Defects in RAG1/2 and 2) Immunodeficiency with radiosensitivity[79]. Defects in RAG1/2 account for 4-20% of all SCID[8], and exhibit a broad spectrum of immunodeficient phenotypes[80,81]. Null mutations in RAG1/2 result in SCID, whereas missense mutations in one or more alleles exhibits partial recombinase activity, resulting in a PID termed Omenn Syndrome[80]. RAG1/2 SCID mutations in T-B-SCID was identified in 1996[82]. Omenn syndrome was first described in 1965, and is characterised by infiltration of the skin by activated lymphoctes. Omenn syndrome results from

leaky mutations in RAG1/2, but has also been associated with mutations in other genes and sometimes, other PIDs itself (reviewed in [79]).

Immunodeficiencies associated with radiosensitivity arise due to mutations in Artemis[83], DNA dependent Protein Kinases (DNA-pk)[84], and deficiency of DNA ligase IV[85], and Cernunnos-XLF[86, 87]. Mutations in these genes result in increased susceptibility to ionising radiation resulting in clinical symptoms similar to those patients with RAG1/2 deficiencies. All the above T-B- SCID have normal levels of NK cells. The entire spectrum of PIDs associated with DNA repair disorders have been previously reviewed[88].

So far, only the most common and severe form of Combined Immunodeficiency, SCID has been reviewed. Other forms of CID include defects in MHC class 1 complex; defects in MHC class 2 complexes, Di George's Syndrome etc. For a complete review of all forms of Cellular Immune defects, including combined Immunodeficiency, please refer [59].

3.2 Predominantly Antibody mediated disorders.

Humoral Immune Defects account for up to 65% of all PIDs described[57]. These defects are among the most commonly observed PIDs in the clinical setting[89, 90, 91]. This is primarily attributed to the fact that patients with some humoral PIDs do not show severe clinical symptoms, when compared to patients

with CID. Patients with humoral immune system deficiencies usually have defects in development, migration or functioning of B cells, and have decreased to no serum levels of circulating immunoglobulins[92]. These patients however, in most cases have a functioning T cell component, which offers a certain degree of protection against intracellular pathogens and fungi. Patients with humoral defects are usually first seen in the clinic with recurrent infections from encapsulated bacteria, particularly *Streptococcus pneumoniae* and *Haemophilus influenzae*[92]. Additionally, while CIDs are severe defects that are often seen during the first few months-years of life, humoral defects can be seen at all ages in the clinical setting. Most cases of humoral immunodeficiency are only observed and diagnosed 7-9 months after birth, once maternal antibody titres decrease and the patients' inability to produce immunoglobulins is noted[93].

Humoral Immune Deficiencies can be further classified into subgroups based on the genetic etiology of defects[56, 92]:

1) Defects in early B cell development.
2) Class Switch Recombination Defects
3) Common Variable Immunodeficiencies (CVID)
4) Selective Immunoglobulin Deficiencies

3.2.1 Defects in early B cell development.

This group of defects includes X-linked agammaglobulinemia (XLA, Bruton's agammaglobulinemia) which was first described by Bruton in 1952[2]. XLA accounts for up to 85% of early onset agammaglobulinemia in humans[8]. XLA symptoms are usually first observed between 3-18 months of age, with mean diagnosis rate in North America being at the age 3 years and median age being 26 months[94]. The disease is clinically manifested by recurrent bacterial infections. Interestingly, XLA is a leaky immunodeficiency, with low numbers of B cells being identified in peripherial blood[8, 92].

Causal mutations of XLA in a cytoplasmic kinase, later called Bruton's tyrosine kinase (Btk) were reported in 1993[95, 96]. Since then, over 600 different mutations have been identified, with more than 90% being insertion or deletion of less than five base pairs, or single base pair substitutions[97, 98]. Btk is involved in the signalling through the B-cell receptor (BCR) and the pre-BCR[99], and is also activated upon binding of respective ligands to IL-5[100] and IL-6[101] receptors on B cells. Due to the large number of mutations, an exact correlation between genotype and phenotype of XLA is not possible[92], although defective Btk results in altered or stagnant downstream cell signalling, resulting in defects in B cell development.

Other forms of early B cell development defects include mutations in the immunoglobulin heavy μ chain gene (IGHM), which accounts for 5% of all early-onset hypogammaglobulinemia in patients. Defects in other essential components of the BCR receptor are more rare[8], and are reviewed in [92].

3.2.2 Class Switch Recombination Defects.

Class switch recombination (CSR) defects, also called Hyper-IgM syndromes are a heterogenous group of disorders. CSR defects are characterised by recurrent bacterial infections, normal to increased serum IgM but low levels of all other isotypes of immunoglobulins[102, 103]. Some CSR defects may also be linked with defects in Somatic Hypermutation. Mature B cells in these patients consist primarily of membrane IgM or IgD, or IgM only, due to defects in class switch to IgG or IgA. These diseases are a rare form of PIDs, with an overall frequency of 1 in 100,000 per live births[103].

Many genetic etiologies have been linked to CSR defects. The most common is CD40 ligand (CD40L) deficiency, which accounts for up to 65% of all Hyper-IgM syndromes[92]. Intriguingly, CD40 ligand deficiencies also result in defects in the T cell compartment, and the latest update on PID classification includes Hyper-IgM defects caused by CD40 ligand deficiency as both a humoral defect and a combined immune defect[56, 104]. Two forms of CSR due to CD40 exist: an X-linked CD40L defect that is characterised by decreased to no CD40L

expression on CD4+ T cells[105, 106, 107, 108], and a rarer autosomal recessive form wherein decreased expression of CD40 is noted on the surface of B cells and monocytes[109]. Symptoms of disease in both these patients are similar, with reduced production of switched classes of immunoglobulins resulting in recurrent and severe infections from bacterial infections. Most patients have decreased memory B cells and reduced frequency of somatic hypermutation[110]. Additionally, due to defects in CD40/CD40L interaction, T cell interaction with monocytes and dendritic cells is altered leading to defects in the cellular mediated immune system as well, resulting in opportunistic infections and liver damage. Prognosis for individuals with this disease is poor[111, 112].

CSR defects also include PIDs that could be termed "pure" humoral defects, in contrast to the cellular and humoral defects observed in CD40/CD40L deficiency[113]. These defects arise due to mutations in genes involved in class switch alone, and include mutations in Activation induced cytidine deaminase (AID) deficiency[114], Uracil N glycosylase (UNG) deficiency[115] and Postmeiotic segregation increased 2 gene (PMS2) deficiency[116]. Genetic etiologies for up to 15% of all Hyper-IgM defects have still not been identified[103]. AID deficiencies account for about 10-15% of CSR defects[92], and both autosomal recessive[114] and autosomal dominant[117] forms have been identified. Individuals with AID deficiencies are associated with recurrent bacterial infections. In one group of patients, retrospective data indicated a significant proportion of showed autoimmune manifestations[118]. Patients with mutations in UNG and PMS2 are

very few, and therefore clinical symptoms and other features are hard to define. However, individuals with mutations in these genes are clinically manifest by recurrent infections[113].

3.2.3 Common Variable Immunodeficiencies

Common Variable Immunodeficiencies (CVID) is an umbrella term for a highly heterogenous set of humoral disorders[11]. CVID is the clinically most prevalent PID, and is observed at a mean frequency of about 1:25000 per live births[55]. A predominantly adult disease, and is often diagnosed during the ages of 25-45 years. Equal number of males and females are affected[55]. It is characterised by decreased serum levels of two or more immunoglobulin isotypes and recurrent sinopulmonary infections and a poor response to vaccines[119]. Additionally, distinct proportions of these patients have splenomegaly, autoimmune phenomena, B cell lymphomas, granulomatous disease and gastrointestinal tract infections[120, 121]. Despite being termed a humoral deficiency, defects in CD4+ and CD8+ T cells[122, 123, 124] and dendritic cells[125] are common and correlate with clinical severity. Additionally, decreased numbers of Natural Killer (NK cells)[126] and Regulatory T cells[127] have also been identified. Currently, classification of CVID is based upon percentage of B cells, with decreased memory B cells correlated to increased clinical severity[128]. Additionally, a method of classification based on both memory B cells and T cells has been proposed[123, 129].

An exact cause of this disease has still not been identified. A majority of CVID cases are sporadic. Between 10-25%[57] of CVID cases are thought to be inherited, with mutations in TACI[130], ICOS[131], CD19[132], BAFF-R[133] and MSH5[134] being associated with the disease. Most familial mutations are autosomal dominant over autosomal recessive[135].

TACI mutations are the most common among CVID patients, and account for up to 10% of all inherited cases[136]. Many TACI mutations have been associated with CVID, with the most common being mutations C104R and A181E. In most CVID patients, these mutations are found in a heterozygous state indicating a dominant negative effect[137]. These mutations are also found in a small proportion of the healthy population as well, suggesting possible development of symptoms at a later age[138]. Other rare mutations have been found, with some being found in equal frequency in both patients and healthy controls, and thus the role of these mutations in CVID is still unclear[136]. TACI is a member of the Tumor Necrosis Factor Receptor (TNF-R) super family, and plays an important role in B cell development and signalling. Expressed on B cells[139], TACI acts as a receptor for B cell activating Factor (BAFF) and A Proliferation Inducing Ligand (APRIL) and plays an important role in B cell activation and survival[140] and Class Switch Recombination[141, 142].

Inducible Costimulator (ICOS) mutations were the first to be described in relation to CVID. ICOS mutations however, account for only 2% of all inherited

CVID cases, and acts in an autosomal recessive manner[57]. ICOS is expressed on the surface of T cells[143], and plays an important role in T cell differentiation and in development of regulatory T cells[143]. ICOS also plays an important role via costimulation in B cell proliferation, differentiation[143] and class switching[144]. Among the nine patients from four unrelated families identified with ICOS mutatons, all of them shared a homozygous deletion of an 1815bp region from the *ICOS* gene resulting in a truncated protein. It was later identified that these patients shared a common ancestor, explaining the common mutation in ICOS[145].

BAFF-R, another member of the TNF Receptor family has also been associated with CVID, albeit in a small number of individuals. BAFF-R acts as a receptor for BAFF, and is important in B cell survival and differentiation. Due to the small number of individuals with BAFF-R mutations, a clear-cut association with CVID is hard to determine. BAFF-R mutations are characterised by low penetrance and wide range of clinical phenotypes, and therefore is thought to be a disease susceptibility gene rather than a disease-causing gene[56, 133].

Only four patients from two unrelated individuals have been identified with mutations in the *CD19* gene, resulting in decreased expression on B cells. It is thought that this decreased expression results in defects in B cell development and signalling[132]. The role of MSH5 in CVID has not been elucidated thoroughly, although it is hypothesised to play a role in the class switch recombination[134]. Defects in TLR7 and TLR9 have been observed in CVID patient B cells[146].

Good's Syndrome is another example of an adult PID with patients most commonly diagnosed between the age of 40 and 70. Individuals with this disease manifest with symptoms similar to those observed in CVID, i.e. hypogammaglobulinemia, decreased to no B cells in peripheral blood and abnormalities in the T cell component. The presence of thymoma along with immunodeficiency is characteristic of patients with Good's Syndrome. Clinical manifestations are similar to those of CVID patients, with increased susceptibility to infections, presence of autoimmune manifestations and diarrhoea. A cause for the disease is unknown[147].

3.2.4 Selective Immunoglobulin Deficiencies

Selective IgA Deficiency (IgAD) is the most common PID observed, with a frequency of up to 1:600 in the general population[148]. It is characterised by decreased levels of serum IgA, but normal levels of all other immunoglobulin classes. Most patients are asymptomatic, but a few experience recurrent infections, autoimmunity and allergies[149]. The pathophysiology of IgAD is not clearly understood, although there appears to be a link between this disease and hereditary forms of CVID[142]. Selective IgG subclass deficiencies are prevalent as well, with no decreases in other classes of immunoglobulins. Both IgAD and selective IgG subclass deficiency are reversible diseases, with age related normals

of IgA and IgG subclasses occurring in patients as they grow older[150]. In some cases however, IgAD can progress into CVID[151].

3.3 Other Well Defined Syndromes

The third class of PIDs, as described in the latest classification from the International Union of Immunological Sciences are those PIDs with defined genetic and clinical basis, but do not fit into either humoral of Combined Immunodeficiencies[56]. In most cases, mutations in genes or proteins that are indirectly involved in haematopoietic cell development are defective, resulting in an immunodeficiency. For example, mutations in genes such as Wiskott Aldrich Syndrome Protein (WASP), which is involved in regulation of actin skeleton in haematopoietic stem cells, or genes involved in thymic development result in Wiskott Aldrich Syndrome[152] and DiGeorge Syndrome respectively[153]. Other PIDs with defects in DNA repair, immuneosseous dysplasias, Hyper-IgE syndromes etc are included in this group[56]. Amongst these, the most common or most well studied are Wiskott-Aldrich syndrome and DiGeorge Syndrome, which will be reviewed in this portion of the literature review.

Wiskott-Aldrich Syndrome is characterised by the classic triad of eczema, thrombocytopenia and immunodeficiency[154], along with small platelets and recurrent and prolonged bleeding [155] and autoimmunity in a substantial proportion of patients[156]. Additionally, IgM levels are normally low, while IgA and IgE

levels are slightly elevated[157]. Identification of mutations in Wiskott-Aldrich Syndrome Protein (WASP) expanded the known clinical symptoms to include classical WAS which is X-linked and fatal without treatment, and milder forms such as X-linked thrombocytopenia (XLT, also attenuated WAS) and congenital neutropenia. Multiple mutations in WASP have been identified, and in general, complete absence of WASP results in classical WAS, while low level of WASP due to missense mutations results in the milder forms[158]. WASP plays an important role as a scaffold protein in cells of the haematopoietic lineage and is involved in trafficking of cells. WASP deficient T cells have defective responses to activation, while WASP is involved in maintainence of B cell homeostasis[152].

DiGeorge Syndrome is associated with a 22q11.2 hemizygous chromosomal deletion[159]. The deletion is present in 1:4000-1:6000 live births, and is often sporadic[160]. However, there have been cases of patients with DiGeorge syndrome who do have this deletion, and individuals with the deletion who suffer from other diseases such as velocardiofacial syndrome[161]. Thus, DiGeorge syndrome is a complex and variable disease, and is clinically diagnosed in patients with cardiac anomalies, defects in the neural crest, learning difficulties, hypocalcemia and mild to moderate immunodeficiency due to defects in thymic development[161]. A number of genes in the region have been linked to symptoms identified in DiGeorge syndrome[162]. The best studied is the gene *Tbx1*. Deletion of *Tbx1* in mice resulted in a phenotype similar to human DiGeorge syndrome, especially in relation to cardiac defects observed in the disease[163, 164]. Later, *Tbx1* mutations

were identified in human patients with DiGeorge syndrome and found to be related to cardiac abnormalities[165].

3.4 Diseases of Immune Dysregulation

Diseases of Immune Dysregulation are an overall rare form of PIDs. In most PIDs included under this heading, defects in pathways involved in lymphocyte signalling are defective, resulting in defects in inflammation, susceptibility to EBV infections, decreased NK cell activity and CD8+ T cell activity etc[8]. The best studied of this group of disorders is the Autoimmune Lymphoproliferative Syndrome (ALPS). ALPS is characterised by non-cancerous lymphoproliferation, lifelong autoimmune disease and an increased risk of Hodgkin and non-Hodkin lymphoma[166]. Individuals with ALPS have defective lymphocyte apoptosis, resulting in altered lymphocyte homeostasis, hypergammaglobulinemia and altered and unusual T cell compartments[167]. Mutations in FAS, FAS ligand, and Caspase10 have been identified in ALPS. Heterozygous FAS germline mutations account for 75% of all ALPS cases, while 20-25% have no known mutations[168]. Mutations in genes involved in apoptotic pathways indicate the importance of apoptosis in the immune system in preventing autoimmune conditions and maintainence of lymphocyte homeostasis. Other disorders of immune dysregulation occur due to mutations in perforin[169], AIRE[170] among others, which lead to deficient cell lysis and defective thymic self-tolerance respectively.

3.5 Diseases of Phagocyte number and function

The most frequent of diseases of phagocyte number and function is Chronic Granulomatous Disease (CGD). First described in the 1950s, CGD has both X-linked and autosomal recessive forms[171]. Originally fatal, increasing knowledge of the disease, therapeutic interventions and improved management has resulted in high survival rates[171]. It has a highly varied frequency, with an estimate of 1:200,000 among live births in a retrospective study of a number of patients[172]. It is clinically manifest by recurrent infections, most commonly from fungal pathogens such as Aspergillus, and bacterial infections from Staphylococcus, Salmonella, Burkholderia, Serratia and Nocardia[172]. Additionally, localised granulomatous lesions from BCG are common[171]. Other symptoms include lymphodenopathy, skin and central nervous system involvement[173]. Overall, patients with the X-linked form of CGD develop symptoms earlier and have a more severe form of disease compared to those patients with the autosomal recessive version[174].

Phagocytes in patients with CGD are unable to generate reactive oxygen species (ROS) against intracellular microorganisms. This defect is due to poor activity of the NADPH oxidase system, due to mutations in one or more of its components, namely gp91phox, p22phox, p47phox and p67phox. Mutations in the *CYBB* gene, which encodes for gp91phox accounts for the X-linked version of the disease, which is the most frequent version of CGD observed (approximately

65%). The second most frequent mutation in CGD is p45phox deficiency which is autosomal recessive and accounts for 30% of all CGD. The remaining two are also autosomal recessive forms of the disease and account for about 10% of CGD. Together, these proteins are involved in the formation of the NADPH oxidase assembly, and mutations in any one of these genes results in loss NADPH assembly formation.[175]

Another interesting PID that is placed under this category is termed Mendelian Susceptibility to Mycobacterial Disease (MSMD)[56]. A highly heterogenous disease, many mutations have been described that lead to increased susceptibility to mycobacterial species including environmental mycobacteria and BCG. Additionally, these individuals are susceptible to other intracellular microorganisms such as salmonella but are relatively resistant to most other bacteria. Mutations in the interferon-gamma signalling pathways have been identified in this disease[176].

3.6 Defects of the innate immune system.

The most striking examples of this group of PIDs include Epidermodysplasia Verruciformis (EV) and Herpes Simplex Encephalitis (HSE). EV has been briefly explained previously in this review. It is an example of a PID caused due to defects in non-haematopoietic cells, and is characterised by increased susceptibility to human papillomavirus due to mutations in the *EVER* genes.

EVER genes are involved in maintainence of zinc homeostasis in epithelium, and act as restriction factors in limiting HPV replication[177]. Mutations in these genes result in uncontrolled proliferation of HPV, resulting in characteristic warts formation and an increased risk of skin malignancies[20].

Another example of an innate immune defect is Herpes Simplex Encephalitis (HSE). It is caused by Herpes Simplex Virus-1 (HSV-1) infection of the Central Nervous System (CNS). HSE is extremely rare, with a frequency of two to four per 1,000,000 people year, and is the most common sporadic viral encephalitis in the Western world[27]. These individuals are characterised by increased susceptibility to HSV-1 infection, but have no increased susceptibility to other pathogens. Mutations in TLR3[178] and UNC93B[179] have been associated with this disease. Mutations in TLR3 are autosomal dominant, and though TLR3 is important in mediating responses to a large number of microbes, the symptoms of HSE suggests TLR3 importance in preventing infection of the CNS[178]. UNC93B is an intracellular protein involved in signalling of anti-viral interferons, and cells obtained from children with HSE who survived indicated enhanced replication of virus and corresponding cell lysis in vitro, both of which were decreased upon exogenous addition of interferon α[179].

3.7 Complement Disorders

Many inherited complement disorders have been described[180, 181]. The complement system is an important constituent of innate immunity, and plays an important role in controlling bacterial infections and in the generation of the classical inflammatory response[182]. Correspondingly, individuals with mutations in the complement system are characterised by increased susceptibility to encapsulated bacteria and autoimmune phenomena. The complement system is complex, and involves many proteins. Deficiencies in the classical pathway, especially of each of the early proteins (C1 complex, C4, C2, C3) are associated with severe disease and development of autoimmune disease similar to systemic lupus eryhtematosus (SLE)[8, 180]. C3 Deficiency is most strongly associated with recurrent and severe infections from encapsulated bacteria such as Neiserria meningitides, Streptococcus pneumoniae and Haemophilus influenzae[183]. Defects of the late terminal complement complex (C5-C9) is associated with severe and invsasive neiserrial infections, but is not associated with autoimmune disease[184]. Defects of the lectin pathway are thought to the most common congenital immunodeficiency, and are associated with increased risk of infections and autoimmune disorders. Defects of the alternate complement pathway are mostly comprised of deficiency of properdin, which is involved in complement activation. It is inherited in an X-linked manner, and is again characterised by increased susceptibility to *N meningiditis*[180, 185].

4. Treatment of Primary Immune Deficiencies

Bacterial infections and fungal infections observed in all PIDs are treated with antibiotics and antifungals respectively. In some patients, antibiotics and antifungals are given as prophylaxis. Interferon-gamma injections are given to patients with severe infections, most commonly those with CGD. Additionally, autoimmune and other inflammatory symptoms are treated by immunosuppressants[8].

Hematopoietic Stem cell Transplant (HCT) was first performed for patients with SCID[186] and WAS[187] in 1968. HCT is now standard of care treatment for patients with SCID, with survival rates of patients ranging from 63-100% with HLA-matched stem cells and 50-77% survival with use of haploidentical or HLA-mismatched donors[188]. HCT is also used currently in the treatment of WAS and CGD, in addition to other rarer PIDs such as X-linked lymphoproliferative syndrome, leukocyte adhesion deficiency and CD40 ligand deficiency[188].

Gene therapy has also been used to treat certain monogenic PIDs. Bone marrow from patients with ADA-SCID and X-SCID were obtained and gene transfer was performed by the use of a retroviral vector. Immunological recovery was observed in these patients, however a signifcant number of individuals who received therapy developed leukaemia-like disorders[7]. In addition to SCID, gene therapy has been attemped in patients with CGD, Leukocyte Adhesion Deficiency

(LAD) and Wiskott Aldrich Syndrome, with mixed results. Current research into this area involves the use of alternate vectors for gene therapy[7].

Intravenous or Subcutaneous immunoglobulins is the treatment of choice for PID patients with significant immunoglobulin deficiencies[189]. It is generated by pooling IgG from plasma of a large number of healthy individuals[190], and infusion of immunoglobulins in patients with PIDs has been shown to increase survival and reduce the frequency of bacterial infections[191]. Additionally, intravenous immunoglobulins have also been shown to have positive effects in patients with inflammation and/or autoimmune conditions[190].

5. Conclusions

The field of PIDs is currently undergoing multiple paradigm shifts. This literature review aims to highlight some of these changes in our understanding of PIDs. PIDs were originally thought to be rare, monogenic, occur predominantly in childhood, be caused due to mutations in the haematopoietic lineage and show a Mendelian mode of inheritance. However, with increased understanding of these diseases and technological advances that aid in detection of these diseases, a novel understanding of the entire spectrum of PIDs is starting to emerge. Additionally, a number of novel PIDs, along with underlying genetic etiologies of previously known and new PIDs are being identified. Improvements in knowledge of PIDs among first line physicians and advances in diagnostic tools

will result in many more PIDs likely to be identified. Research into this heterogenous group of disorders will not only lead to improvements in therapeutic and treatment options, but research into causes of PIDs would also inform us about the functioning of the human immune system (References for the literature review portion of this thesis are on page 68)

V Manuscript 1

Adult Primary Immune Deficiency

Authors:

Bharat T Srinivasa[1, 2,] Reza Alizadehfar[1], Martin Desrosiers[3], Joseph Shuster[1], Nitika Pant Pai[4], Christos M Tsoukas[1, 2]

[1] Division of Allergy and Clinical Immunology, McGill University Health Center, Montreal, Canada

[2] Department of Microbiology and Immunology, McGill University, Montreal, Canada

[3] Department of Otorhinolaryngology, McGill University Health Center, Montreal, Canada

[4] Division of Clinical Epidemiology, McGill University Health Centre, Montreal, Canada.

1. Connector:

As indicated in the literature review, multiple paradigm shifts in our understanding of the field of Primary Immunodeficiency are occurring. One of the most interesting is the identification of PIDs in adult patients. To date, there has been a paucity of description of exclusively adult cohort of PID patients. The manuscript in the following pages represents Canada's first description of PID patients, and the largest number of adult-only PID patients to be described.

2. Contribution of authors:

Bharat T Srinivasa and Christos M Tsoukas were involved in data collection, data analysis and writing up the manuscript. Reza Alizadehfar, Martin Desrosiers, Joseph Shuster and Christos M Tsoukas were responsible for diagnosis and treatment of patients. Nitika Pant Pai was involved in verifying the epidemiological nature of the manuscript.

3. Introduction

Primary Immune Deficiencies (PIDs) are caused by inherited defects in the immune system. Until recently, PIDs were thought to be primarily due to defects in cells of the haematopoietic lineage[1]. The first traditionally recognized PID, described by Bruton in 1952 was X-linked agammaglobulinemia[2]. Our knowledge of PIDs is evolving, and PIDs are now known to result from defects in the non-haematopoietic arm of the immune system[1]. Epidermodysplasia Verruciformis (EV) is characterized by increased susceptibility to Human Papillomavirus (HPV) [Reviewed in [3]] was identified in 1922[4] and only classified as a PID in the early 2000s upon discovery of mutations in EVER1 and EVER2 genes[5]. These genes are known to be important in the maintenance of zinc homeostasis in the epithelium and for restriction factors limiting HPV replication[6]. Over 130 PIDs have so far described[7, 8].

Immune Deficiencies were originally thought to be rare in adults. However our views on these illnesses are changing, as recent studies have shown that up to 1 in 1200 people in the United States are diagnosed with some form of PID[9]. The most common PID in the western world is IgA deficiency, with an incidence in Caucasians of up to 1:600[10]. Prevalence of this disorder varies between ethnicities, with lower prevalence among Orientals[10]. Fortunately, this disorder is mostly sub clinical in presentation. The most frequently diagnosed clinically recognisable adult PID is Common Variable Immune Deficiency

(CVID) and has an ethnicity-based prevalence of 1:25000 among Caucasians[10]. Higher rates of PIDs are usually observed in populations with high consanguinity rates or among genetically isolated populations [7].

Primary Immune Deficiencies are a highly variable set of diseases that can present with a diverse range of clinical and immune phenotypes as well as underlying genotypes. In addition to genetic and clinically defined syndromes, subtle and often subclinical immune defects can occur in adults. These are expressed with variable penetrance and may go unrecognized in general clinical practice. Individuals are often only recognized as immune deficient following extensive investigations for unforeseen, unusual, recurrent or severe infections. As a result of the relative rarity, clinical heterogeneity, and limited diagnostic tools, misdiagnoses in clinical settings and potential misclassification of patients often occur. These factors have resulted in delays in making PID diagnoses and ultimately in the initiation of appropriate therapies [11].

PIDs were previously classified according to broad categories of defects found in the humoral, cellular or combined components of the immune system or with phagocytic or complement abnormalities. Recently with the discovery of many causal gene defects, the ability to further classify these patients has improved. A more precise classification can now be envisioned based not only on the presence or absence of immune cells but at a molecular level that defines receptor or cell signalling pathway defects[12]. Abnormalities in humoral immunity

account for over 50% of PIDs[13], and individuals with these defects are more susceptible to encapsulated bacterial infections while T cell anomalies account for a lower number of PIDs, and are considerably more severe and often fatal[14]. With severe T cell deficiency, death occurs as a result of opportunistic infections and neoplasms. Children with these defects can also present with a failure to thrive[14].

A majority of adults with primary immune deficiencies are not diagnosed or treated early in their course[15]. This has been due to a paucity of prevailing knowledge of these illnesses in the health care community and because of insufficient awareness about the existence of such conditions in adults.

In the year 2000, the Immune Deficiency Treatment Centre (IDTC) of McGill University undertook to establish a dedicated clinic for adults with PID. The reasons were: 1) the improved management and increased survival of children with PIDs made it necessary to provide ongoing transition care as they entered adulthood 2) an unmet need was identified to diagnose, manage and treat individuals with adult onset PIDs and 3) to increase awareness of the existence of adults with these disorders within the medical community.

We conducted a retrospective chart review of 381 consecutive patients referred to us with a suspected preliminary diagnosis of immune deficiency. Our study objectives were first, to analyze and describe the observed spectrum of

immune defects in adult patients referred to us and second, to determine the presumptive pre-referral diagnostic accuracy of immune deficiency.

4. Materials and Methods

We conducted a chart review of all adult patients sequentially presenting to our centre with a suspected diagnosis of Immune deficiency during a 10 year period (Jan 1, 2000-Dec 31, 2009). Standard clinical and diagnostic criteria were used to identify primary and secondary immune deficiencies. Institutional approval for the study was obtained from the McGill University Health Centre.

Our study inclusion criteria were:

1. Adults (18 years and older) at the time of initial evaluation of suspected immunodeficiency. 2. Availability of demographic data, reasons for referral, clinical presentation, the initial pre-evaluation and the final post-evaluation diagnosis. 3. Availability of specific diagnostic laboratory data including immunophenotyping data, assays of immune function and associated genetic testing. Individuals were excluded: 1) if they did not meet any of the above criteria, 2) if they had HIV infection, 3) were on immunosuppressive therapy.

Diagnosis: The diagnoses were made by any of the three clinical immunologists working at the centre and were based on standard criteria set by the European Society for Immunodeficiencies and the Pan-American Group for

Immunodeficiency (ESID/PAGID)[16]. Patients were diagnosed based on the following specific criteria: **X-Linked Agammaglobulinemia**- male patients with less than 2% CD19+ B cells in which the following have occurred: 1) onset of recurrent bacterial infections in the first 5 years of life, 2) Serum IgG, IgM and IgA levels significantly decreased (at least 2 SD below mean for age), 3) absent isohemaggluttinins and/or poor response to vaccines and 4) exclusion of other causes of hypogammaglobulinemia[16]. **Common Variable Immune Deficiency**-patients having a marked decrease (at least 2 SD below mean for age) in serum IgG and two other major isotype of immunoglobulin, clinical onset of immunodeficiency defined by recurrent or severe infection at greater than 2 years of age, absent isohemaggluttinins and/or poor response to vaccine and exclusion of other defined causes of hypogammagloblulinemia[16]. **IgA Deficiency**-patients with serum IgA levels 2 SD below the mean for the age but having normal serum IgG and IgM levels, and with the exclusion of other defined causes of hypogammaglobulinemia[16]. **Hypogammglobulinemia**- low levels (2 SD below mean) of IgG and IgM or only IgM respectively, while maintaining normal serum levels of other immunoglobulin isotypes with a history of infection that were not severe or recurrent and an exclusion of other defined causes of hypogammglobulinemia[16]. **Specific Pneumococcal Antibody Deficiency-** having selective or specific deficiency in antibody responses to pneumococcal antibody. **Selective Antibody Deficiency** having decreased subclasses of IgG antibody. **Wiskott Aldrich Syndrome (WAS)** in males with congenital thrombocytopenia (<70,000 platelets/mm^3) and one of the following: eczema, abnormal antibody

response to polysaccharide antigens, recurrent infections, autoimmune disease, lymphoma, leukaemia and/or brain tumour[16]. **Job's syndrome** in individuals with high serum IgE levels (>2000 IU/ml), recurrent staphylococcal skin abscesses, eczema and pneumonia[17]. **Idiopathic CD4+ T-lymphocytopenia-** low CD4+ T cell counts (CD4+ T cells less than 20% of total lymphocytes), no serological evidence of HIV infection and exclusion of other causes of immunodeficiency or therapy induced T-cell depletion [18, 19]. **Good's Syndrome**: thymoma, hypogammaglobulinemia and recurrent infections[20]. **Chronic NK cell lymphocytosis (CNKL):** persistent NK cell excess for a period of up to 6 months, flow cytometry determination of NK cell phenotype and absence of confounding clinical disease such as NK cell lymphoma[21]. **Chronic granulomatous disease**: at least one test indicating abnormal functioning of the phagocytic NADPH oxidase system, or abnormal intracellular bactericidal activity of their phagocytic cells, as described in the National Registry for Patients with CGD in the US[22]

Secondary immune deficiency was diagnosed when the immune defect resulted from a co-morbid condition or as a therapeutic side effect. Other clinical conditions that related to autoimmune and autoinflammatory states were noted and defined as follows: **Immune Thrombocytic Purpura:** by patient clinical history, physical examination, blood count and examination of the peripheral smear to exclude other causes of thrombocytopenia[23]. **Behcet's disease**: patient having recurrent oral ulcerations and two of genital ulcerations, eye lesions and/or skin lesions[24]. **Familial Mediterranean Fever:** according to the Livneh criteria

for the disease[25]. **Tumor Necrosis Factor (TNF) Receptor Associated Periodic Syndrome (TRAPS)** was identified based on history, physical examination and detection of low levels of serum type I TNF receptor when measured between attacks, and by identification of mutations in the *TNFRSFIA* gene[26].

5. Results

Demographic profile: a wide spectrum of clinical phenotypes was evaluated. They were grouped based on the existence or absence of an immune deficiency.

Table 1 profiles the characteristics of all referred patients.

Table 1: Description of patients referred to the IDTC

Characteristics	**Total patients** n=381	Immune Deficiency			
		Primary n= 196 (51%)	**Secondary** n=55 (14%)	**Undetermined** n=14 (4%)	**None** n=116 (31%)
Gender					
Male	39% (149)	43% (84)	42% (23)	29% (4)	33% (38)
Female	61% (232)	57% (112)	58% (32)	71% (10)	67% (78)
Median Age (yrs)	45 (18-89)	44 (18-83)	53 (18-79)	49 (20-76)	46 (18-89)
Male	48 (18-89)	42 (18-80)	55 (19-79)	30 (20-51)	50 (18-89)
Female	45 (18-88)	45 (19-83)	42 (18-79)	57 (20-76)	42 (18-88)
Ethnicity					
Caucasian	97% (372)	97% (191)	96% (53)	100% (14)	98% (114)
Black	1% (3)	2% (3)	0% (0)	0% (0)	0% (0)
Oriental	2% (6)	1% (2)	4% (2)	0% (0)	2% (2)

Of the 251 patients with clinically diagnosed immune deficiency disease, 196 had a PID and 55 had secondary immune deficiency. The most commonly

observed primary immune defect was: CVID (37%, n=72), followed by, IgA deficiency (13%, n=25), hypogammaglobulinemia (11% n=21), and Idiopathic CD4+ T lymphopenia (ICL) (7%, n=14). Despite extensive investigation, 14 patients were diagnosed with presumptive PIDs based on their clinical history of recurrent and /or severe infections. The exact nature of their defects however, remains undetermined as immunophenotyping and functional assays were normal. The list of PID defining diseases and other immune disorders observed is indicated in table

Table 2: Diagnoses of patients with suspected immunodeficiency

Immunodeficiency Diseases/Syndromes				
Immune System Defect	Diagnosis	N =	Median Age	Range
Humoral immunity	**Defined**			
	Common Variable Immunodeficiency	72	38	18-80
	IgA Deficiency	25	45	18-79
	X-linked Agammaglobulinemia	5	21	19-72
	Specific Pneumococcal Antibody Deficiency	4	53	20-75
	Selective Antibody Deficiency	3	33	33-40
	Job's Syndrome	1	19	-
	Hyper IgM Syndrome	1	46	-
	Novel			
	Hypogammaglobulinemia	27	52	21-72
	Low Circulating B Cells with bacterial infections	12	67	40-76
Cell mediated immunity	**Defined**			
	T Cell Deficiency with Chronic Candidiasis	11	48	30-65
	Abnormal Cytokine Receptor	1	20	-
	Wiskott Aldrich Syndrome	1	23	-
	Other			
	Idiopathic CD4+ T-cell Lymphocytopenia	14	55	24-83
	Idiopathic CD8+ T-cell Lymphocytopenia	1	39	-
Combined B and T cell defects	Good's Syndrome	4	59	58-63
	Combined Immunodeficiency(DOCK 8 deficiency)	1	19	-
Innate immunity	**Defined**			
	Natural Killer Cells Defect	6	38	27-48
	Novel			
	Chronic Natural Killer Cell Lymphocytosis	2	63	55-70

	(CNKCL)			
Phagocytic disorders	Neutropenia Chronic Granulomatous Disease	4 1	51 33	50-57 -
Total		**196**		

Table 3: Diagnoses of patients without Primary Immunodeficiencies

Other Immune System Defects/Disorders				
Autoimmune Diseases	Autoimmune Syndromes	7	44	22-65
	Systemic Lupus Erythrematosus	5	39	18-79
	Immune Thrombocytic Purpura	2	43	32-53
Auto-inflammatory Diseases	Familial Mediterranean Fever	6	22	18-38
	Tumour Necrotic Factor Receptor-associated Periodic Syndrome (TRAPS)	1	53	-
Secondary immune deficiency states	Chronic Lymphocytic Leukaemia with hypogammaglobulinemia	5	71	49-74
	Lymphoma	2	71	40-71
	Post-splenectomy	2	38	22-54
	Sarcoidosis	2	55	54-56
	Secondary Hypergammaglobulinemia	2	40	31-49
	Sjogren's Syndrome	2	54	53-55
	Waldenstrom's Disease	2	74	68-79
	Behcet's Syndrome	1	54	-
	Kaposi's Sarcoma- non HIV related	1	56	-
	Monoclonal Gammopathy	1	40	-
	Secondary Dysgammaglobulinemia	1	56	-
	Scelorising Cholangiitis	1	41	-
	Thymoma	1	75	-
Other Hematologic	Secondary Lymphopenia	7	48	20-76
	Secondary Leukopenia	2	44	19-68
Others	Secondary Hypereosinophilia	2	69	67-70
Total		**55**		
Unknown	Clinical Immune Deficiency – Undetermined cause	14	49	20-76

We have diagnosed 251 of all the patients referred to us with a clinical phenotype consistent with an Immune Deficiency, and 78% (196/251) of these had Primary Immune Deficiency. Out of the 251 individuals, 208 were diagnosed

at our centre resulting in a referral accuracy rate of 55%. The remaining patients (43) were referred to us with prior PID diagnoses. These patients were either transitioned from paediatric centres or transferred for reasons of relocation. Overall, we could accurately classify 95% of all PID, and fail to classify 5%.

Five deaths were reported in the study period. All these patients had severe T cell defects and were male. These were: a 21 year old with a DOCK 8 (Dedicator Of Cytokinesis 8) deficiency who died from metastatic anal carcinoma, a 70 year old with Good's Syndrome who died from multifocal leucoencepholopathy (PML). Two patients with severe T cell abnormalities and had experienced multiple opportunistic infections. The first a 71 year old who died from leukemia and the other, a 27 year old from infectious complications following a bone marrow transplantation. The last individual with ICL was 25 years old when he died from overwhelming sepsis.

Following the above analysis and tabulation of diagnoses, all the symptoms that triggered each patient to be referred to us by their physicians were described. This data is presented in table 4. A large number of patients referred to us had chronic sinusitis (n=53, 14%), chronic bronchitis (n=18, 5%) and recurrent pneumonia (n=17, 5%). In otherwise healthy adults these clinical problems are common, however they can also be associated with CVID. To determine if one or more of these clinical phenotypes was more common in those with immune

deficiency, we characterised the proportion of patients that were later diagnosed as having CVID versus those that were classified as immunologically normal.

A significantly larger number of individuals referred for chronic sinusitis and recurrent pneumonia were diagnosed as having CVID ($p<0.01$). This was not the case for those referred for chronic bronchitis or bronchiectasis. Viral infections were significantly more common as reasons for referral ($p<0.01$) in the non-immune deficient (non-ID) patients, and included patients affected by recurrent viral exanthem, mononucleosis, HCV, HSV and Herpes Zoster viruses

Other reasons for referral included; fatigue, chronic diarrhea, recurrent otitis, chronic skin and fungal infections. 26 non-ID patients and 6 CVID patients were also observed to have non-infectious systemic disorders and listed in Table 4 by system. In patients with CVID these were idiopathic thrombocytopenic purpura (ITP) or hemolytic anemia. As listed in Table 4, 26 patients with known rheumatologic, haematologic and dermatologic disorders known to be associated with immune deficiency were evaluated and no evidence of immune deficiency noted. None had history of recurrent infection. Additionally, some of the patients referred to us for suspected immune deficiency included those with other co-morbidities; such as multiple sclerosis, lung cancer and diabetes mellitus. Dermatological infections included facial abscesses and eczema. Non-infectious dermatologic disorders were noted exclusively in the non immune deficient patients and this finding was statistically significantly when compared to the

CVID group. Of the CVID patients, 78% (56%) had sino-pulmonary infections as their primary presenting phenotype. The remaining 22% had other predominant clinical features.

Table 4: Reason for referral of patients without any PID vs. those with CVID.

Reason for Referral		Non PID	CVID	P
Infections	Chronic Sinusitis	21	32	<0.01
	Recurrent Pneumonia	5	12	<0.01
	Chronic Bronchitis	9	9	NS
	Chronic Bronchiectasis	2	0	NS
	Chronic Otitis	0	3	NS
	Chronic Diarrhoea	5	4	NS
	Skin infection	9	1	NS
	Chronic Urinary Tract Infection	2	0	NS
	Fungal Infection	2	1	NS
	Recurrent Viral Infections	17	2	<0.01
	Recurrent Respiratory Infection	4	1	NS
System Disorder	Rheumatological	5	0	NS
	Haematological	5	6	NS
	Dermatological	9	0	<0.05
	Endocrinological	1	0	NS
	Neurological	2	0	NS
	Other systems	3	0	NS
Other Reasons	Laboratory Abnormalities	5	0	NS
	Chronic Fatigue	9	1	NS
	Malignancy	1	0	NS
Total number of patients		**116**	**72**	

NS- Not Significant
Stats test: Fischer's exact test
Software: Stata 10.1.

The majority of individuals referred to us come from adult Allergists and Clinical Immunologists (n=89), followed by family practitioners (n= 51),

otorhinolaryngologists (n=32), pulmonologists (n=24), Pediatric Clinical Immunologists (n=23), Rheumatologists (n=20), Endocrinologists (n=20), Hematologists (n=18), Gastroenterologists (n=10) Internists (n=8), Infectious Disease specialists (n=6), Cardiologists(n=2) and Gynecologists (n=2).

6. Discussion

In this report, we attempt to characterise the infectious complications and systemic pathologies observed in patients referred to us with suspected immunodeficiency. This is the first known study to describe a large number of adults with Primary Immune Deficiency followed in a single center in Canada.

Primary Immune Deficiencies are relatively rare diseases; although some experts argue that advances in modern medicine may have masked the full clinical presentation of certain immune deficits allowing these individuals to live relatively normal lives[1]. There is no single clinical phenotype for Primary Immune Deficiency. This results in a high number of under- or misdiagnoses in general clinical practice. Frequencies of PIDs in adults range from 1:600 to 1:500,000, depending on the type of PID and ethnicity of the affected individuals. The highest frequency of diagnosed PIDs is observed in Caucasians.

In paediatric cohorts of PID most patients are male[27, 28, 29, 30, 31]. Interestingly our patients were mostly female. This could be attributed to the

nature of our study population: many PIDs, especially those that are X-linked result in more severe clinical manifestations and a failure to survive in male children. Therefore, our population, and possibly other adult PID cohorts would be expected to show a higher proportion of females in comparison to paediatric cohorts.

Due to the spectrum of possible inherent defects in the immune system, the clinical presentation varies for most adults with PIDs. The clinical phenotype may be dependent on the underlying infectious pathogen, the frequency and severity of infection or the expression of autoimmune processes. The causative microbial agent should trigger a pattern of clinical investigations that follows a particular arm of the immune system. For instance, individuals with defects in humoral immunity are more prone to bacterial infections, whereas severe viral or fungal infections are frequently noted with T-cell defects. Adults with defective cell-mediated immunity or those with combined immune defects are rarer, at higher risk of death since they are more likely to present with severe and often life threatening infections with opportunistic pathogens. Indeed, all five patients that died during the study period were males and had severe cell mediated immune deficiencies.

Most adult PIDs observed in daily clinical practise are those with humoral immune defects and are not generally fatal. The functioning T cell arm of the immune system may compensate and offer partial protection in these individuals.

On the other hand, T cell defects and combined immunodeficiencies are rarely seen in general practice, have a more severe clinical phenotype, and may result in death during childhood. This may explain our findings where 77% of all our adult PID patients had a humoral defect, 14.3% were diagnosed with a T cell mediated immune defect and only 2.6% had combined deficiencies in both arms of the immune system.

A majority of our patients that had clinical immune deficiency were diagnosed with CVID (n=72, 37%). IgA deficiency, which has been observed to be more common than CVID generally has a sub clinical manifestation and was understandably under-represented in our cohort (n=25, 13%). Referring physicians do not often request evaluation of individuals with low serum IgA and no history of infections. Also asymptomatic individuals are not likely to have serum immunoglobulin quantitation.

We noted two novel groups of individuals with humoral immune defects. The first consisted of 27 individuals with infrequent and non-severe bacterial infections that had hypogammaglobulinemia. The second group consisted of 12 individuals (mostly women) with a history of recurrent bacterial infections, low B cell numbers (two SD below mean) but with normal serum levels of immunogloblins. Additionally, two novel groups of patients with cell-mediated abnormalities were observed. With CD4 and/or CD8 T cell lymphopenia. Idiopathic CD4+ T cell Lymphocytopenia is a rare disease[32] and almost always

seen in adults. Fourteen individuals (7%) were diagnosed with this pathology, reflecting the unique nature of our adult cohort and underlying the heterogeneity of adults diagnosed with PID. Despite the low and persistent absolute CD4 T cell numbers, only one of these patients developed opportunistic infections. Most of our patients with ICL were diagnosed during cell phenotyping whereas many reported in the literature are diagnosed following the appearance of opportunistic infections.

Additionally, we also describe 116 patients, classified as the Non Immunodeficient (Non-ID) group. Following evaluation they did not have either primary or secondary immune deficiency. We wished to determine if any of the pre-evaluation characteristics in the clinical history of the non-ID patients would be different from those with PID. Since CVID was the most frequently encountered PID we compared the profile of infections by site and by organ system. A significantly larger number of patients referred with Chronic Sinusitis and Recurrent Pneumonia had CVID ($p<0.01$), thus underscoring the importance of these disorders in making one suspect immunodeficiency. Recurrent lower respiratory infections, including bronchitis and pneumonia, are among the most common presenting clinical manifestations of those with CVID. With equal numbers of patients in each group, no differences between groups were noted for those having bronchitis. Additionally, a significantly large number of individuals characterised as non-ID were initially referred to us with recurrent viral infections. Of note only 35% of the 72 CVID patients had quantitative

immunoglobulin assessment prior to referral. Given the overall high pre-referral presumptive diagnosis of immune deficiency, it was surprising that quantitative immunoglobulin testing to diagnose CVID was not undertaken by the referring physicians for the majority of patients. The testing is easily and widely accessible and affordable, yet was not frequently ordered.

Despite considerable efforts to determine the exact cause of recurrent infections in 14 patients (4% of the study population) we were unable to provide specific diagnoses for this group and their syndromes remain unclassified.

Even with our increasing knowledge of PIDs and the ongoing identification of underlying genetic mechanisms, many patients still suffer due to poor treatment and misdiagnoses[33]. There appears to be a disconnect between research into the disease at the laboratory level and treatment of the disease at a clinical level. The J project, set up in March 2004 in Eastern and Central Europe was initiated in order to ensure adequate transfer of knowledge between health professionals, scientific societies and patient associations. Established by the European Society for Immunodeficiency (ESID) due to poor reporting of these diseases in these populations, the project has had reasonable success in increasing awareness of the diseases among medical practitioners, community leaders and the general population in Eastern Europe[33]. The establishment of such a project in Canada would greatly help in ensuring dissemination of knowledge regarding PIDs.

Lastly, there is currently a paradigm shift in our understanding of PIDs. These were previously thought to be rare and to affect only children in a recessive, Mendelian manner. We are now starting to understand the incomplete penetrance of PID phenotypes as well as their complex and variable genotypes and furthermore to identify adults with PIDs more frequently. (Reviewed by Casanova and Abel[1]) Adult PIDs represent a novel entity and further research into these heterogenous disorders is required in order to ensure proper diagnosis and timely treatment is provided.

7. References:

1. Casanova, J.L. & Abel, L. Primary immunodeficiencies: a field in its infancy. *Science* **317**, 617-9 (2007).
2. Bruton, O. Agammaglobulinemia. *Pediatrics* **9**, 722-728 (1952).
3. Gewirtzman, A., Bartlett, B. & Tyring, S. Epidermodysplasia verruciformis and human papilloma virus. *Current Opinion in Infectious Diseases* **21**, 141-146 (2008).
4. Lewandowsky, F. & Lutz, W. A case of a previously undescribed skin disease (epidermodysplasia verruciformis) [in German]. *Arch Dermat Syphilol* 193-203 (1922).
5. Ramoz, N. et al. Mutations in two adjacent novel genes are associated with epidermodysplasia verruciformis. *Nat. Genet* **32**, 579-581 (2002).

6. Lazarczyk, M. et al. Regulation of cellular zinc balance as a potential mechanism of EVER-mediated protection against pathogenesis by cutaneous oncogenic human papillomaviruses. *J. Exp. Med* **205**, 35-42 (2008).
7. Notarangelo, L.D. 15. Primary immunodeficiencies. *J Allergy Clin Immunol* (2009).doi:10.1016/j.jaci.2009.07.053
8. Notarangelo, L. et al. Primary immunodeficiency diseases: an update. *J Allergy Clin Immunol* **114**, 677-87 (2004).
9. Boyle, J.M. & Buckley, R.H. Population prevalence of diagnosed primary immunodeficiency diseases in the United States. *J Clin Immunol* **27**, 497-502 (2007).
10. Hammarstrom, L., Vorechovsky, I. & Webster, D. Selective IgA deficiency (SIgAD) and common variable immunodeficiency (CVID). *Clin Exp Immunol* **120**, 225-31 (2000).
11. Yarmohammadi, H., Estrella, L., Doucette, J. & Cunningham-Rundles, C. Recognizing primary immune deficiency in clinical practice. *Clin Vaccine Immunol* **13**, 329-32 (2006).
12. Cooper, M.A., Pommering, T.L. & Korányi, K. Primary immunodeficiencies. *Am Fam Physician* **68**, 2001-8 (2003).
13. Woroniecka, M. & Ballow, M. Office evaluation of children with recurrent infection. *Pediatr. Clin. North Am* **47**, 1211-1224 (2000).
14. Buckley, R. Primary cellular immunodeficiencies. *Journal of Allergy and Clinical Immunology* **109**, 747-757 (2002).
15. Mansouri, D. et al. Primary immune deficiencies presenting in adults: seven

years of experience from Iran. *J. Clin. Immunol* **25**, 385-391 (2005).

16. Conley, M.E., Notarangelo, L.D. & Etzioni, A. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). *Clin Immunol* **93**, 190-7 (1999).
17. Grimbacher, B., Holland, S.M. & Puck, J.M. Hyper-IgE syndromes. *Immunol Rev* **203**, 244-50 (2005).
18. Mukherjee, A., Lodha, R. & Kabra, S. Idiopathic CD4+ T-cell lymphocytopenia. *Indian Journal of Pediatrics* doi:10.1007/s12098-009-0002-8
19. Smith, D.K., Neal, J.J. & Holmberg, S.D. Unexplained opportunistic infections and CD4+ T-lymphocytopenia without HIV infection. An investigation of cases in the United States. The Centers for Disease Control Idiopathic CD4+ T-lymphocytopenia Task Force. *N Engl J Med* **328**, 373-9 (1993).
20. Kelleher, P. & Misbah, S.A. What is Good's syndrome? Immunological abnormalities in patients with thymoma. *J Clin Pathol* **56**, 12-16 (2003).
21. Rabbani, G.R., Phyliky, R.L. & Tefferi, A. A long-term study of patients with chronic natural killer cell lymphocytosis. *Br J Haematol* **106**, 960-6 (1999).
22. Winkelstein, J.A. et al. Chronic granulomatous disease. Report on a national registry of 368 patients. *Medicine (Baltimore)* **79**, 155-169 (2000).
23. George, J.N. et al. Idiopathic thrombocytopenic purpura: a practice guideline developed by explicit methods for the American Society of Hematology.

Blood **88**, 3-40 (1996).

24. O'Neill, T.W., Rigby, A.S., Silman, A.J. & Barnes, C. Validation of the International Study Group criteria for Behçet's disease. *Br J Rheumatol* **33**, 115-7 (1994).
25. Livneh, A. et al. Criteria for the diagnosis of familial mediterranean fever. *Arthritis & Rheumatism* **40**, 1879-1885 (1997).
26. Masson, C. et al. Tumor necrosis factor receptor-associated periodic syndrome (TRAPS): definition, semiology, prognosis, pathogenesis, treatment, and place relative to other periodic joint diseases. *Joint Bone Spine* **71**, 284-290 (2004).
27. Rezaei, N. et al. Frequency and clinical manifestations of patients with primary immunodeficiency disorders in Iran: update from the Iranian Primary Immunodeficiency Registry. *J. Clin. Immunol* **26**, 519-532 (2006).
28. Grumach, A.S. et al. Brazilian report on primary immunodeficiencies in children: 166 cases studied over a follow-up time of 15 years. *J Clin Immunol* **17**, 340-5 (1997).
29. Hayakawa, H., Iwata, T., Yata, J. & Kobayashi, N. Primary immunodeficiency syndrome in Japan. I. Overview of a nationwide survey on primary immunodeficiency syndrome. *J. Clin. Immunol* **1**, 31-39 (1981).
30. Stray-Pedersen, A., Abrahamsen, T.G. & Frøland, S.S. Primary immunodeficiency diseases in Norway. *J. Clin. Immunol* **20**, 477-485 (2000).
31. Reda, S.M., Afifi, H.M. & Amine, M.M. Primary immunodeficiency diseases in Egyptian children: a single-center study. *J. Clin. Immunol* **29**, 343-351

(2009).

32. Walker, U.A. & Warnatz, K. Idiopathic CD4 lymphocytopenia. *Curr Opin Rheumatol* **18**, 389-95 (2006).

33. Maródi, L. & Casanova, J. Primary immunodeficiency diseases: the J Project. *The Lancet* **373**, 2179-2181 (2009).

VI Conclusion

The field of Primary Immune Deficiencies is a rapidly growing area of clinical and fundamental research. With improvements in detection techniques, and an increasing awareness of these diseases among clinicians, novel PIDs and genetic aetiologies are being described more frequently. With increasing numbers of PIDs, trends are starting to emerge in our understanding of these highly heterogenous sets of diseases. Amongst these, is the concept of the changing paradigms of primary immune deficiencies, which has been highlighted in this thesis. Among the most interesting paradigm shifts is the understanding that PIDs can affect adults. With improvements in therapeutic interventions for children with PIDs, many more infants are surviving into adulthood. Thus research into Primary Immune Deficiencies with adults serves two purposes: 1) it allows continued monitoring of children with PIDs as they progress into adulthood and 2) it allows researchers to study individuals who develop PIDs during adulthood. The study of these aspects of PIDs will lead to a clearer understanding of the functioning of the immune system, and help provide therapeutic options to patients of all ages with primary immunodeficiencies.

VII References (Literature Review)

1. Fleisher, T.A. Back to Basics: Primary Immune Deficiencies: Windows into the Immune System. *Pediatrics in Review* **27**, 363-372 (2006).
2. Bruton, O.C. Agammaglobulinemia. *Pediatrics* **9**, 722-728 (1952).
3. Casanova, J., Fieschi, C., Zhang, S. & Abel, L. Revisiting human primary immunodeficiencies. *Journal of Internal Medicine* **264**, 115-127 (2008).
4. Porta, F. et al. Stem cell transplantation for primary immunodeficiencies. *Bone Marrow Transplant* **41 Suppl 2**, S83-86 (2008).
5. Filipovich, A. Hematopoietic cell transplantation for correction of primary immunodeficiencies. *Bone Marrow Transplant* **42 Suppl 1**, S49-S52 (2008).
6. Casanova, J. & Abel, L. Primary Immunodeficiencies: A Field in Its Infancy. *Science* **317**, 617-619 (2007).
7. Kohn, D.B. Update on gene therapy for immunodeficiencies. *Clin Immunol* (2010).doi:10.1016/j.clim.2009.12.003
8. Notarangelo, L.D. Primary immunodeficiencies. *J Allergy Clin Immunol* (2009).doi:10.1016/j.jaci.2009.07.053
9. Latiff, A.H.A. & Kerr, M.A. The clinical significance of immunoglobulin A deficiency. *Ann. Clin. Biochem* **44**, 131-139 (2007).
10. Renzo, M., Pasqui, A. & Auteri, A. Common variable immunodeficiency: a review. *Clinical and Experimental Medicine* **3**, 211-217 (2004).
11. Deane, S., Selmi, C., Naguwa, S.M., Teuber, S.S. & Gershwin, M.E. Common variable immunodeficiency: etiological and treatment issues. *Int. Arch. Allergy Immunol* **150**, 311-324 (2009).
12. Lee, P.P.W. & Lau, Y. Primary immunodeficiencies: "new" disease in an old

country. *Cell. Mol. Immunol* **6**, 397-406 (2009).

13. Knerr, V. & Grimbacher, B. Primary immunodeficiency registries. *Curr Opin Allergy Clin Immunol* **7**, 475-480 (2007).
14. Casanova, J. & Abel, L. The human model: a genetic dissection of immunity to infection in natural conditions. *Nat Rev Immunol* **4**, 55-66 (2004).
15. Casanova, J. & Abel, L. Inborn errors of immunity to infection: the rule rather than the exception. *J. Exp. Med.* **202**, 197-201 (2005).
16. Quintana-Murci, L., Alcaïs, A., Abel, L. & Casanova, J. Immunology in natura: clinical, epidemiological and evolutionary genetics of infectious diseases. *Nat. Immunol* **8**, 1165-1171 (2007).
17. Lewandowsky, F. & Lutz, W. Ein Fall einer bisher nicht beschriebenen Hauterkrankung (Epidermodysplasia verruciformis). *Archives of Dermatological Research* **141**, 193-203 (1922).
18. Ruiter, M. & van Mullem, P.J. Demonstration by electronmicroscopy of an intranuclear virus in epidermodysplasia verruciformis. *J. Invest. Dermatol* **47**, 247-252 (1966).
19. Baker, H. Epidermodysplasia verruciformis with electron microscopic demonstration of virus. *Proc. R. Soc. Med* **61**, 589-591 (1968).
20. Lutzner, M.A. Epidermodysplasia verruciformis. An autosomal recessive disease characterized by viral warts and skin cancer. A model for viral oncogenesis. *Bull Cancer* **65**, 169-182 (1978).
21. Ramoz, N., Rueda, L.A., Bouadjar, B., Favre, M. & Orth, G. A susceptibility locus for epidermodysplasia verruciformis, an abnormal predisposition to

infection with the oncogenic human papillomavirus type 5, maps to chromosome 17qter in a region containing a psoriasis locus. *J. Invest. Dermatol* **112**, 259-263 (1999).

22. Ramoz, N. et al. Evidence for a nonallelic heterogeneity of epidermodysplasia verruciformis with two susceptibility loci mapped to chromosome regions 2p21-p24 and 17q25. *J. Invest. Dermatol* **114**, 1148-1153 (2000).
23. Ramoz, N. et al. Mutations in two adjacent novel genes are associated with epidermodysplasia verruciformis. *Nat Genet* **32**, 579-581 (2002).
24. Orth, G. Genetics of epidermodysplasia verruciformis: Insights into host defense against papillomaviruses. *Seminars in Immunology* **18**, 362-374 (2006).
25. CHAPEL, H., GEHA, R. & ROSEN, F. Primary immunodeficiency diseases: an update. *Clinical & Experimental Immunology* **132**, 9-15 (2003).
26. Fischer, A. Primary immunodeficiency diseases: an experimental model for molecular medicine. *Lancet* **357**, 1863-1869 (2001).
27. Sancho-Shimizu, V. et al. Genetic susceptibility to herpes simplex virus 1 encephalitis in mice and humans. *Curr Opin Allergy Clin Immunol* **7**, 495-505 (2007).
28. Würzner, R., Orren, A. & Lachmann, P.J. Inherited deficiencies of the terminal components of human complement. *Immunodefic Rev* **3**, 123-147 (1992).
29. Vanhollebeke, B. et al. Human Trypanosoma evansi infection linked to a

lack of apolipoprotein L-I. *N. Engl. J. Med* **355**, 2752-2756 (2006).

30. Casanova, J. & Abel, L. Primary Immunodeficiencies: A Field in Its Infancy. *Science* **317**, 617-619 (2007).

31. Nickel, R.G. et al. Determination of Duffy genotypes in three populations of African descent using PCR and sequence-specific oligonucleotides. *Hum. Immunol* **60**, 738-742 (1999).

32. Miller, L.H., Mason, S.J., Dvorak, J.A., McGinniss, M.H. & Rothman, I.K. Erythrocyte receptors for (Plasmodium knowlesi) malaria: Duffy blood group determinants. *Science* **189**, 561-563 (1975).

33. Miller, L.H., Mason, S.J., Clyde, D.F. & McGinniss, M.H. The resistance factor to Plasmodium vivax in blacks. The Duffy-blood-group genotype, FyFy. *N. Engl. J. Med* **295**, 302-304 (1976).

34. Tournamille, C., Colin, Y., Cartron, J.P. & Le Van Kim, C. Disruption of a GATA motif in the Duffy gene promoter abolishes erythroid gene expression in Duffy-negative individuals. *Nat. Genet* **10**, 224-228 (1995).

35. Samson, M. et al. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature* **382**, 722-725 (1996).

36. Liu, R. et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* **86**, 367-377 (1996).

37. Lindesmith, L. et al. Human susceptibility and resistance to Norwalk virus infection. *Nat. Med* **9**, 548-553 (2003).

38. Bacchelli, C., Buckridge, S., Thrasher, A.J. & Gaspar, H.B. Translational mini-review series on immunodeficiency: molecular defects in common variable immunodeficiency. *Clin. Exp. Immunol* **149**, 401-409 (2007).

39. Aslam, A. & Chapel, H. Dissecting the group of common variable immunodeficiency disorders. *Clin. Infect. Dis* **49**, 1339-1340 (2009).

40. Miller, E.N. et al. Genome-wide scans for leprosy and tuberculosis susceptibility genes in Brazilians. *Genes Immun* **5**, 63-67 (2004).

41. Siddiqui, M.R. et al. A major susceptibility locus for leprosy in India maps to chromosome 10p13. *Nat. Genet* **27**, 439-441 (2001).

42. Tosh, K. et al. A region of chromosome 20 is linked to leprosy susceptibility in a South Indian population. *J. Infect. Dis* **186**, 1190-1193 (2002).

43. Mira, M.T. et al. Chromosome 6q25 is linked to susceptibility to leprosy in a Vietnamese population. *Nat. Genet* **33**, 412-415 (2003).

44. Mira, M.T. et al. Susceptibility to leprosy is associated with PARK2 and PACRG. *Nature* **427**, 636-640 (2004).

45. Alter, A., Alcaïs, A., Abel, L. & Schurr, E. Leprosy as a genetic model for susceptibility to common infectious diseases. *Hum. Genet* **123**, 227-235 (2008).

46. Alcaïs, A., Mira, M., Casanova, J., Schurr, E. & Abel, L. Genetic dissection of immunity in leprosy. *Curr. Opin. Immunol* **17**, 44-48 (2005).

47. Schurr, E., Alcaïs, A., de Léséleuc, L. & Abel, L. Genetic predisposition to leprosy: A major gene reveals novel pathways of immunity to Mycobacterium leprae. *Semin. Immunol* **18**, 404-410 (2006).

48. Lawrence, T. et al. Autosomal-dominant primary immunodeficiencies. *Curr. Opin. Hematol* **12**, 22-30 (2005).

49. Kawai, T. & Malech, H.L. WHIM syndrome: congenital immune deficiency disease. *Curr. Opin. Hematol* **16**, 20-26 (2009).

50. Hernandez, P.A. et al. Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. *Nat. Genet* **34**, 70-74 (2003).

51. Holland, S.M. et al. STAT3 mutations in the hyper-IgE syndrome. *N. Engl. J. Med* **357**, 1608-1619 (2007).

52. Engelhardt, K.R. et al. Large deletions and point mutations involving the dedicator of cytokinesis 8 (DOCK8) in the autosomal-recessive form of hyper-IgE syndrome. *J. Allergy Clin. Immunol* **124**, 1289-1302.e4 (2009).

53. Minegishi, Y. et al. Human tyrosine kinase 2 deficiency reveals its requisite roles in multiple cytokine signals involved in innate and acquired immunity. *Immunity* **25**, 745-755 (2006).

54. Freeman, A., Davis, J., Hsu, A., Holland, S. & Puck, J. Autosomal Dominant Hyper IgE syndrome. *GeneReviews [Internet]* (2010).

55. Cunningham-Rundles, C. & Knight, A.K. Common variable immune deficiency: reviews, continued puzzles, and a new registry. *Immunol. Res* **38**, 78-86 (2007).

56. Notarangelo, L.D. et al. Primary immunodeficiencies: 2009 update. *J. Allergy Clin. Immunol* **124**, 1161-1178 (2009).

57. Park, M.A., Li, J.T., Hagan, J.B., Maddox, D.E. & Abraham, R.S. Common

variable immunodeficiency: a new look at an old disease. *Lancet* **372**, 489-502 (2008).

58. Fischer, A. et al. Severe combined immunodeficiency. A model disease for molecular immunology and therapy. *Immunol. Rev* **203**, 98-109 (2005).
59. Buckley, R.H. Primary cellular immunodeficiencies. *J. Allergy Clin. Immunol* **109**, 747-757 (2002).
60. Cunningham-Rundles, C. & Ponda, P.P. Molecular defects in T- and B-cell primary immunodeficiency diseases. *Nat. Rev. Immunol* **5**, 880-892 (2005).
61. Puck, J.M. X-linked severe combined immunodeficiency. *Primary Immunodeficiency diseases: a molecular and genetic approach* 123-136 (2007).
62. Puck, J.M. Population-based newborn screening for severe combined immunodeficiency: steps toward implementation. *J. Allergy Clin. Immunol* **120**, 760-768 (2007).
63. Noguchi, M. et al. Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans. *Cell* **73**, 147-157 (1993).
64. Kovanen, P.E. & Leonard, W.J. Cytokines and immunodeficiency diseases: critical roles of the gamma(c)-dependent cytokines interleukins 2, 4, 7, 9, 15, and 21, and their signaling pathways. *Immunol. Rev* **202**, 67-83 (2004).
65. Davis, J. & Puck, J.M. X-linked Severe Combined Immunodeficiency. *GeneReviews [Internet]* (2005).
66. Russell, S.M. et al. Mutation of Jak3 in a patient with SCID: essential role of

Jak3 in lymphoid development. *Science* **270**, 797-800 (1995).

67. Suzuki, K. et al. Janus kinase 3 (Jak3) is essential for common cytokine receptor gamma chain (gamma(c))-dependent signaling: comparative analysis of gamma(c), Jak3, and gamma(c) and Jak3 double-deficient mice. *Int. Immunol* **12**, 123-132 (2000).

68. Puel, A., Ziegler, S.F., Buckley, R.H. & Leonard, W.J. Defective IL7R expression in T(-)B(+)NK(+) severe combined immunodeficiency. *Nat. Genet* **20**, 394-397 (1998).

69. Kung, C. et al. Mutations in the tyrosine phosphatase CD45 gene in a child with severe combined immunodeficiency disease. *Nat. Med* **6**, 343-345 (2000).

70. Tchilian, E.Z. et al. A deletion in the gene encoding the CD45 antigen in a patient with SCID. *J. Immunol* **166**, 1308-1313 (2001).

71. de Saint Basile, G. et al. Severe combined immunodeficiency caused by deficiency in either the delta or the epsilon subunit of CD3. *J. Clin. Invest* **114**, 1512-1517 (2004).

72. Roberts, J.L. et al. T-B+NK+ severe combined immunodeficiency caused by complete deficiency of the CD3zeta subunit of the T-cell antigen receptor complex. *Blood* **109**, 3198-3206 (2007).

73. Shiow, L.R. et al. Severe combined immunodeficiency (SCID) and attention deficit hyperactivity disorder (ADHD) associated with a Coronin-1A mutation and a chromosome 16p11.2 deletion. *Clin. Immunol* **131**, 24-30 (2009).

74. Hershfield, M. Adenosine Deaminase Deficiency. *GeneReviews [Internet]* (2009).

75. Scott, C.R., Chen, S.H. & Giblett, E.R. Detection of the carrier state in combined immunodeficiency disease associated with adenosine deaminase deficiency. *J. Clin. Invest* **53**, 1194-1196 (1974).

76. Ochs, H.D. et al. Adenosine-deaminase deficiency and severe combined immunodeficiency syndrome. *Lancet* **1**, 1393-1394 (1973).

77. Nyhan, W.L. Disorders of purine and pyrimidine metabolism. *Mol. Genet. Metab* **86**, 25-33 (2005).

78. Giblett, E.R. ADA and PNP deficiencies: how it all began. *Ann. N. Y. Acad. Sci* **451**, 1-8 (1985).

79. de Villartay, J. V(D)J recombination deficiencies. *Adv. Exp. Med. Biol* **650**, 46-58 (2009).

80. Villa, A. et al. V(D)J recombination defects in lymphocytes due to RAG mutations: severe immunodeficiency with a spectrum of clinical presentations. *Blood* **97**, 81-88 (2001).

81. Niehues, T., Perez-Becker, R. & Schuetz, C. More than just SCID--the phenotypic range of combined immunodeficiencies associated with mutations in the recombinase activating genes (RAG) 1 and 2. *Clin. Immunol* **135**, 183-192 (2010).

82. Schwarz, K. et al. RAG mutations in human B cell-negative SCID. *Science* **274**, 97-99 (1996).

83. Nicolas, N. et al. A human severe combined immunodeficiency (SCID)

condition with increased sensitivity to ionizing radiations and impaired V(D)J rearrangements defines a new DNA recombination/repair deficiency. *J. Exp. Med* **188**, 627-634 (1998).

84. van der Burg, M. et al. A DNA-PKcs mutation in a radiosensitive T-B-SCID patient inhibits Artemis activation and nonhomologous end-joining. *J. Clin. Invest* **119**, 91-98 (2009).

85. Riballo, E. et al. Identification of a defect in DNA ligase IV in a radiosensitive leukaemia patient. *Curr. Biol* **9**, 699-702 (1999).

86. Buck, D. et al. Cernunnos, a novel nonhomologous end-joining factor, is mutated in human immunodeficiency with microcephaly. *Cell* **124**, 287-299 (2006).

87. Ahnesorg, P., Smith, P. & Jackson, S.P. XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. *Cell* **124**, 301-313 (2006).

88. Slatter, M.A. & Gennery, A.R. Primary immunodeficiencies associated with DNA-repair disorders. *Expert Rev Mol Med* **12**, e9 (2010).

89. Stray-Pedersen, A., Abrahamsen, T.G. & Frøland, S.S. Primary immunodeficiency diseases in Norway. *J. Clin. Immunol* **20**, 477-485 (2000).

90. Ryser, O., Morell, A. & Hitzig, W.H. Primary immunodeficiencies in Switzerland: first report of the national registry in adults and children. *J. Clin. Immunol* **8**, 479-485 (1988).

91. Rezaei, N. et al. Frequency and clinical manifestations of patients with

primary immunodeficiency disorders in Iran: update from the Iranian Primary Immunodeficiency Registry. *J. Clin. Immunol* **26**, 519-532 (2006).

92. Conley, M.E. et al. Primary B cell immunodeficiencies: comparisons and contrasts. *Annu. Rev. Immunol* **27**, 199-227 (2009).
93. Moise, A. et al. Primary immunodeficiencies of the B lymphocyte. *J Med Life* **3**, 60-63 (2010).
94. Conley, M.E. & Howard, V. Clinical findings leading to the diagnosis of X-linked agammaglobulinemia. *J. Pediatr* **141**, 566-571 (2002).
95. Tsukada, S. et al. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell* **72**, 279-290 (1993).
96. Vetrie, D. et al. The gene involved in X-linked agammaglobulinaemia is a member of the src family of protein-tyrosine kinases. *Nature* **361**, 226-233 (1993).
97. Conley, M.E. et al. Genetic analysis of patients with defects in early B-cell development. *Immunol. Rev* **203**, 216-234 (2005).
98. Väliaho, J., Smith, C.I.E. & Vihinen, M. BTKbase: the mutation database for X-linked agammaglobulinemia. *Hum. Mutat* **27**, 1209-1217 (2006).
99. Mohamed, A.J. et al. Bruton's tyrosine kinase (Btk): function, regulation, and transformation with special emphasis on the PH domain. *Immunological Reviews* **228**, 58-73 (2009).
100. Sato, S. et al. IL-5 receptor-mediated tyrosine phosphorylation of SH2/SH3-containing proteins and activation of Bruton's tyrosine and Janus 2 kinases. *J. Exp. Med* **180**, 2101-2111 (1994).

101. Matsuda, T. et al. Association and activation of Btk and Tec tyrosine kinases by gp130, a signal transducer of the interleukin-6 family of cytokines. *Blood* **85**, 627-633 (1995).

102. Notarangelo, L.D., Duse, M. & Ugazio, A.G. Immunodeficiency with hyper-IgM (HIM). *Immunodefic Rev* **3**, 101-121 (1992).

103. Durandy, A., Taubenheim, N., Peron, S. & Fischer, A. Pathophysiology of B-cell intrinsic immunoglobulin class switch recombination deficiencies. *Adv. Immunol* **94**, 275-306 (2007).

104. Kracker, S., Gardes, P., Mazerolles, F. & Durandy, A. Immunoglobulin class switch recombination deficiencies. *Clin. Immunol* **135**, 193-203 (2010).

105. Allen, R.C. et al. CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. *Science* **259**, 990-993 (1993).

106. Aruffo, A. et al. The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. *Cell* **72**, 291-300 (1993).

107. DiSanto, J.P., Bonnefoy, J.Y., Gauchat, J.F., Fischer, A. & de Saint Basile, G. CD40 ligand mutations in x-linked immunodeficiency with hyper-IgM. *Nature* **361**, 541-543 (1993).

108. Korthäuer, U. et al. Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper-IgM. *Nature* **361**, 539-541 (1993).

109. Ferrari, S. et al. Mutations of CD40 gene cause an autosomal recessive form of immunodeficiency with hyper IgM. *Proc. Natl. Acad. Sci. U.S.A* **98**, 12614-12619 (2001).

110. Agematsu, K. et al. Absence of IgD-CD27(+) memory B cell population in

X-linked hyper-IgM syndrome. *J. Clin. Invest* **102**, 853-860 (1998).

111. Levy, J. et al. Clinical spectrum of X-linked hyper-IgM syndrome. *J. Pediatr* **131**, 47-54 (1997).

112. Winkelstein, J.A. et al. The X-linked hyper-IgM syndrome: clinical and immunologic features of 79 patients. *Medicine (Baltimore)* **82**, 373-384 (2003).

113. Davies, E.G. & Thrasher, A.J. Update on the hyper immunoglobulin M syndromes. *Br J Haematol* (2010).doi:10.1111/j.1365-2141.2010.08077.x

114. Revy, P. et al. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). *Cell* **102**, 565-575 (2000).

115. Imai, K. et al. Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination. *Nat. Immunol* **4**, 1023-1028 (2003).

116. Péron, S. et al. Human PMS2 deficiency is associated with impaired immunoglobulin class switch recombination. *J. Exp. Med* **205**, 2465-2472 (2008).

117. Imai, K. et al. Analysis of class switch recombination and somatic hypermutation in patients affected with autosomal dominant hyper-IgM syndrome type 2. *Clin. Immunol* **115**, 277-285 (2005).

118. Quartier, P. et al. Clinical, immunologic and genetic analysis of 29 patients with autosomal recessive hyper-IgM syndrome due to Activation-Induced Cytidine Deaminase deficiency. *Clin. Immunol* **110**, 22-29 (2004).

119. Scharenberg, AM, Hannibal, MC, Torgerson T, Ochs, H.D. & Rawlings, D.J. Common Variable Immune Deficiency Overview. *GeneReviews [Internet]* (2006).

120. Cunningham-Rundles, C. & Bodian, C. Common variable immunodeficiency: clinical and immunological features of 248 patients. *Clin. Immunol* **92**, 34-48 (1999).

121. Aghamohammadi, A. et al. Clinical and immunological features of 65 Iranian patients with common variable immunodeficiency. *Clin. Diagn. Lab. Immunol* **12**, 825-832 (2005).

122. Carbone, J., Sarmiento, E., Micheloud, D., Rodríguez-Molina, J. & Fernández-Cruz, E. Elevated levels of activated CD4 T cells in common variable immunodeficiency: association with clinical findings. *Allergol Immunopathol (Madr)* **34**, 131-135 (2006).

123. Lanio, N., Sarmiento, E., Gallego, A. & Carbone, J. Immunophenotypic profile of T cells in common variable immunodeficiency: is there an association with different clinical findings? *Allergol Immunopathol (Madr)* **37**, 14-20 (2009).

124. Viallard, J. et al. CD8+HLA-DR+ T lymphocytes are increased in common variable immunodeficiency patients with impaired memory B-cell differentiation. *Clin. Immunol* **119**, 51-58 (2006).

125. Yong, P.F.K. et al. Selective deficits in blood dendritic cell subsets in common variable immunodeficiency and X-linked agammaglobulinaemia but not specific polysaccharide antibody deficiency. *Clin. Immunol* **127**, 34-

42 (2008).

126. Aspalter, R.M., Sewell, W.A., Dolman, K., Farrant, J. & Webster, A.D. Deficiency in circulating natural killer (NK) cell subsets in common variable immunodeficiency and X-linked agammaglobulinaemia. *Clin. Exp. Immunol* **121**, 506-514 (2000).
127. Melo, K.M. et al. A decreased frequency of regulatory T cells in patients with common variable immunodeficiency. *PLoS ONE* **4**, e6269 (2009).
128. Wehr, C. et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. *Blood* **111**, 77-85 (2008).
129. Giovannetti, A., Pierdominici, M. & Aiuti, F. T-cell homeostasis: the dark(ened) side of common variable immunodeficiency. *Blood* **112**, 446; author reply 446-447 (2008).
130. Salzer, U. et al. Mutations in TNFRSF13B encoding TACI are associated with common variable immunodeficiency in humans. *Nat. Genet* **37**, 820-828 (2005).
131. Grimbacher, B. et al. Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. *Nat. Immunol* **4**, 261-268 (2003).
132. van Zelm, M.C. et al. An antibody-deficiency syndrome due to mutations in the CD19 gene. *N. Engl. J. Med* **354**, 1901-1912 (2006).
133. Warnatz, K. et al. B-cell activating factor receptor deficiency is associated with an adult-onset antibody deficiency syndrome in humans. *Proc. Natl. Acad. Sci. U.S.A* **106**, 13945-13950 (2009).
134. Sekine, H. et al. Role for Msh5 in the regulation of Ig class switch

recombination. *Proc. Natl. Acad. Sci. U.S.A* **104**, 7193-7198 (2007).

135. Schroeder, H.W., Schroeder, H.W. & Sheikh, S.M. The complex genetics of common variable immunodeficiency. *J. Investig. Med* **52**, 90-103 (2004).
136. Lee, J.J., Ozcan, E., Rauter, I. & Geha, R.S. Transmembrane activator and calcium-modulator and cyclophilin ligand interactor mutations in common variable immunodeficiency. *Curr Opin Allergy Clin Immunol* **8**, 520-526 (2008).
137. Garibyan, L. et al. Dominant-negative effect of the heterozygous C104R TACI mutation in common variable immunodeficiency (CVID). *J. Clin. Invest* **117**, 1550-1557 (2007).
138. Castigli, E. et al. Reexamining the role of TACI coding variants in common variable immunodeficiency and selective IgA deficiency. *Nat. Genet* **39**, 430-431 (2007).
139. Salzer, U. & Grimbacher, B. TACItly changing tunes: farewell to a yin and yang of BAFF receptor and TACI in humoral immunity? New genetic defects in common variable immunodeficiency. *Curr Opin Allergy Clin Immunol* **5**, 496-503 (2005).
140. Bossen, C. et al. TACI, unlike BAFF-R, is solely activated by oligomeric BAFF and APRIL to support survival of activated B cells and plasmablasts. *Blood* **111**, 1004-1012 (2008).
141. Castigli, E. et al. TACI and BAFF-R mediate isotype switching in B cells. *J. Exp. Med* **201**, 35-39 (2005).
142. Castigli, E. & Geha, R.S. TACI, isotype switching, CVID and IgAD.

Immunol. Res **38**, 102-111 (2007).

143. Yong, P.F.K., Salzer, U. & Grimbacher, B. The role of costimulation in antibody deficiencies: ICOS and common variable immunodeficiency. *Immunol. Rev* **229**, 101-113 (2009).

144. McAdam, A.J. et al. ICOS is critical for CD40-mediated antibody class switching. *Nature* **409**, 102-105 (2001).

145. Salzer, U. et al. ICOS deficiency in patients with common variable immunodeficiency. *Clin. Immunol* **113**, 234-240 (2004).

146. Yu, J.E. et al. Toll-like receptor 7 and 9 defects in common variable immunodeficiency. *J. Allergy Clin. Immunol* **124**, 349-356, 356.e1-3 (2009).

147. Kelesidis, T. & Yang, O. Good's syndrome remains a mystery after 55 years: A systematic review of the scientific evidence. *Clin. Immunol* **135**, 347-363 (2010).

148. Hammarstrom, L., Vorechovsky, I. & Webster, D. Selective IgA deficiency (SIgAD) and common variable immunodeficiency (CVID). *Clin Exp Immunol* **120**, 225-31 (2000).

149. Janzi, M. et al. Selective IgA deficiency in early life: association to infections and allergic diseases during childhood. *Clin. Immunol* **133**, 78-85 (2009).

150. Kutukculer, N., Karaca, N.E., Demircioglu, O. & Aksu, G. Increases in serum immunoglobulins to age-related normal levels in children with IgA and/or IgG subclass deficiency. *Pediatr Allergy Immunol* **18**, 167-173 (2007).

151. Aghamohammadi, A. et al. Progression of selective IgA deficiency to common variable immunodeficiency. *Int. Arch. Allergy Immunol* **147**, 87-92 (2008).

152. Thrasher, A.J. & Burns, S.O. WASP: a key immunological multitasker. *Nat. Rev. Immunol* **10**, 182-192 (2010).

153. Lischner, H.W. & Huff, D.S. T-cell deficiency in diGeorge syndrome. *Birth Defects Orig. Artic. Ser* **11**, 16-21 (1975).

154. Kolluri, R. et al. Identification of WASP mutations in patients with Wiskott-Aldrich syndrome and isolated thrombocytopenia reveals allelic heterogeneity at the WAS locus. *Hum. Mol. Genet* **4**, 1119-1126 (1995).

155. Sullivan, K.E., Mullen, C.A., Blaese, R.M. & Winkelstein, J.A. A multiinstitutional survey of the Wiskott-Aldrich syndrome. *J. Pediatr* **125**, 876-885 (1994).

156. Dupuis-Girod, S. et al. Autoimmunity in Wiskott-Aldrich syndrome: risk factors, clinical features, and outcome in a single-center cohort of 55 patients. *Pediatrics* **111**, e622-627 (2003).

157. Thrasher, A.J. New insights into the biology of Wiskott-Aldrich syndrome (WAS). *Hematology Am Soc Hematol Educ Program* 132-138 (2009).doi:10.1182/asheducation-2009.1.132

158. Ochs, H.D. Mutations of the Wiskott-Aldrich Syndrome Protein affect protein expression and dictate the clinical phenotypes. *Immunol. Res* **44**, 84-88 (2009).

159. Chapelle, A., Herva, R., Koivisto, M. & Aula, P. A deletion in chromosome

22 can cause digeorge syndrome. *Human Genetics* **57**, 253-256 (1981).

160. Botto, L.D. et al. A population-based study of the 22q11.2 deletion: phenotype, incidence, and contribution to major birth defects in the population. *Pediatrics* **112**, 101-107 (2003).
161. Kobrynski, L.J. & Sullivan, K.E. Velocardiofacial syndrome, DiGeorge syndrome: the chromosome 22q11.2 deletion syndromes. *Lancet* **370**, 1443-1452 (2007).
162. Antshel, K.M., Kates, W.R., Roizen, N., Fremont, W. & Shprintzen, R.J. 22q11.2 deletion syndrome: genetics, neuroanatomy and cognitive/behavioral features keywords. *Child Neuropsychol* **11**, 5-19 (2005).
163. Merscher, S. et al. TBX1 is responsible for cardiovascular defects in velo-cardio-facial/DiGeorge syndrome. *Cell* **104**, 619-629 (2001).
164. Jerome, L.A. & Papaioannou, V.E. DiGeorge syndrome phenotype in mice mutant for the T-box gene, Tbx1. *Nat. Genet* **27**, 286-291 (2001).
165. Yagi, H. et al. Role of TBX1 in human del22q11.2 syndrome. *Lancet* **362**, 1366-1373 (2003).
166. Bleesing, JJH, Johnson J & Zhang, K Autoimmune Lymphoproliferative Syndrome. *GeneReviews [Internet]* (2009).
167. Sneller, M.C. et al. Clincal, immunologic, and genetic features of an autoimmune lymphoproliferative syndrome associated with abnormal lymphocyte apoptosis. *Blood* **89**, 1341-1348 (1997).
168. Bleesing, J.J.H. Autoimmune lymphoproliferative syndrome (ALPS). *Curr.*

Pharm. Des **9**, 265-278 (2003).

169. Stepp, S.E. et al. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. *Science* **286**, 1957-1959 (1999).
170. Heino, M. et al. APECED mutations in the autoimmune regulator (AIRE) gene. *Hum. Mutat* **18**, 205-211 (2001).
171. Holland, S.M. Chronic granulomatous disease. *Clin Rev Allergy Immunol* **38**, 3-10 (2010).
172. Winkelstein, J.A. et al. Chronic granulomatous disease. Report on a national registry of 368 patients. *Medicine (Baltimore)* **79**, 155-169 (2000).
173. Movahedi, M. et al. Chronic granulomatous disease: a clinical survey of 41 patients from the Iranian primary immunodeficiency registry. *Int. Arch. Allergy Immunol* **134**, 253-259 (2004).
174. van den Berg, J.M. et al. Chronic granulomatous disease: the European experience. *PLoS ONE* **4**, e5234 (2009).
175. Jurkowska, M., Bernatowska, E. & Bal, J. Genetic and biochemical background of chronic granulomatous disease. *Arch. Immunol. Ther. Exp. (Warsz.)* **52**, 113-120 (2004).
176. Al-Muhsen, S. & Casanova, J. The genetic heterogeneity of mendelian susceptibility to mycobacterial diseases. *J. Allergy Clin. Immunol* **122**, 1043-1051; quiz 1052-1053 (2008).
177. Lazarczyk, M. et al. Regulation of cellular zinc balance as a potential mechanism of EVER-mediated protection against pathogenesis by cutaneous oncogenic human papillomaviruses. *J. Exp. Med* **205**, 35-42 (2008).

178. Zhang, S. et al. TLR3 deficiency in patients with herpes simplex encephalitis. *Science* **317**, 1522-1527 (2007).

179. Casrouge, A. et al. Herpes simplex virus encephalitis in human UNC-93B deficiency. *Science* **314**, 308-312 (2006).

180. Botto, M. et al. Complement in human diseases: Lessons from complement deficiencies. *Mol. Immunol* **46**, 2774-2783 (2009).

181. Unsworth, D.J. Complement deficiency and disease. *J. Clin. Pathol* **61**, 1013-1017 (2008).

182. Dunkelberger, J.R. & Song, W. Complement and its role in innate and adaptive immune responses. *Cell Res* **20**, 34-50 (2010).

183. Pickering, M.C., Botto, M., Taylor, P.R., Lachmann, P.J. & Walport, M.J. Systemic lupus erythematosus, complement deficiency, and apoptosis. *Adv. Immunol* **76**, 227-324 (2000).

184. Würzner, R., Orren, A. & Lachmann, P.J. Inherited deficiencies of the terminal components of human complement. *Immunodefic Rev* **3**, 123-147 (1992).

185. Daha, M.R. Role of complement in innate immunity and infections. *Crit. Rev. Immunol* **30**, 47-52 (2010).

186. Gatti, R., Meuwissen, H., Allen, H., Hong, R. & Good, R. IMMUNOLOGICAL RECONSTITUTION OF SEX-LINKED LYMPHOPENIC IMMUNOLOGICAL DEFICIENCY. *The Lancet* **292**, 1366-1369 (1968).

187. Bach, F., Albertini, R., Joo, P., Anderson, J. & Bortin, M. BONE-

MARROW TRANSPLANTATION IN A PATIENT WITH THE WISKOTT-ALDRICH SYNDROME. *The Lancet* **292**, 1364-1366 (1968).

188. de la Morena, M.T. & Gatti, R.A. A history of bone marrow transplantation. *Immunol Allergy Clin North Am* **30**, 1-15 (2010).
189. Ballow, M. et al. Immunodeficiencies. *Clin. Exp. Immunol* **158 Suppl 1**, 14-22 (2009).
190. Hartung, H. et al. Clinical applications of intravenous immunoglobulins (IVIg) - beyond immunodeficiencies and neurology. *Clinical & Experimental Immunology* **158**, 23-33 (2009).
191. Wood, P. Primary antibody deficiencies: recognition, clinical diagnosis and referral of patients. *Clin Med* **9**, 595-599 (2009).