Primary Immunodeficiency: The Israeli Connection

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KEY WORDS: immunodeficiency, Israel, pediatrics, primary immunodeficiency (PID)

IMAJ 2018; 20: 703–706

The field of primary immunodeficiency (PID) is quite young in medicine. Until the early 1950s there were no treatment options for patients with immunodeficiency and no laboratory techniques were in use. With the invention of protein-electrophoresis technology, the first case of agammaglobulinemia was described in 1952. Since then, the field has increased dramatically and the first cases of successful bone marrow transplantation, and later gene therapy, were made in patients with PID. Currently, more than 350 different genes have been found to be defective in cases of immune deficiency and the list increases every month [1].

In Israel, in the first years after its independence, there was no interest in immunodeficiency. The founder of the field in Israel was the late Prof. Stanley Levine [Figure 1].

Figure 1. Prof. Levine and his wife at their wedding in 1948

After earning a medical degree in South Africa, he arrived in Israel on the day of the declaration of the State of Israel – 14 May 1948. He was sent directly to the frontline and opened a field hospital in Gedera in the Negev. After the war, he moved to Tiberius and headed the department of pediatrics. In 1956, he was sent to study pediatric immunology at Johns Hopkins University in Baltimore, MD, USA. When he returned, he became the director of pediatrics at Kaplan Hospital in Rehovot and started his research on ataxia telangiectasia and Down syndrome. He was able to show the cellular immune defects in these two syndromes by applying new techniques that he learned in the United States [2,3].

Prof. Zeev Hadzel, who replaced Prof. Levine at the pediatric immunology unit at Kaplan Hospital, belongs to the second generation of pediatric immunologists in Israel. He continued to research Down syndrome and studied the effect of thymic humoral factor in various forms of PID [4]. Later, he devoted most of his time to studying the first cases of human immunodeficiency virus (HIV) in Israel, together with Prof. Zvi Bentwitz [5].

Two other prominent researchers concluded the second generation of pediatric immunologists in Israel. The first, Prof. Zvi Spirer, began his research in the early 1970s. He was interested in studying infectious agents. Already in the late 1960s he was taking care of patients with severe combined immunodeficiency and hypogammaglobulinemia, and he treated the country's first case of bare lymphocyte syndrome. Later he focused his interest on phagocytosis and chemotaxis and the importance of the spleen, as well as the tuffsin hormone, for the immune system [6]. He also treated a patient with combined immunodeficiency with a thymic transplant but unfortunately after several weeks the transplant was rejected (personal communication).

The third prominent physician in the group is Prof. Jastin Passwell. Like Prof. Levine, he finished his medical training in South Africa and immigrated to Israel during the early 1970s. Later, he attended Harvard University, USA, and completed a fellowship in pediatric immunology under the mentorship of Dr. Fred Rosen and Dr. Harvey Colten, two well-known researchers in the field. After returning to Sheba Medical Center, he established a pediatric immunology research laboratory. He was interested in complement defects [6] and chronic granulomatous disease, and performed the first bone marrow transplantation for this condition in Israel. He then attempted to develop a new vaccine against dysentery [7], research he continued until his death at the age of 63 years.

In the early 1980s, four young pediatricians (all retired by now) created the third generation. Prof. Benzi Garti was at Schneider Children's Medical Center and devoted his time to the study of PID and the immune defects in glycogen storage disease [8]. Prof. Yacov Levi went to Soroka Medical Center in Beer Sheba and wrote a seminal paper on hyper IgM syndrome [9]. He headed the immunological clinic for many years. Prof. Menachem Slezinger established the immunology service at Barzilai Medical Center in Ashkelon and headed the Israeli center for complement disorders there [10]. The last one in the group is Prof. Amos Etzioni from Rambam Healthcare Campus in Haifa, where the first Jeffery Modell Foundation (JMF) center for PID in Israel was created. His research focus was mainly on new forms of leukocyte adhesion deficiency syndromes [11].

The list cannot be completed without mentioning two other pediatricians who,

although not trained in immunology, contributed significantly to the field of PID in Israel. The first was Prof. Baruch Wolach, who finished his pediatric hematology fellowship under the late Prof. Rina Zaizov and continued to study neutrophil function in the laboratory of Dr. Larry Boxer in the United States. After his return, he opened a laboratory for leukocyte function at Meir Medical Center in Kfar Saba and he dedicated his time to study many neutrophil defects, mainly chronic granulomatous disease (CGD) [12]. The second was Prof. Gidi Rechavi from Sheba Medical Center, who also was trained as a pediatric hemato-oncologist. Under his leadership, the immunological pediatric unit expanded and became a well-known laboratory in the field. He published many articles in the field of PID, discovered several new entities, and was involved with bone marrow transplantation in patients with PID. Rechavi and his student Prof. Amos Toren were among the pioneers in the application of preimplantation genetic diagnosis (PGD) for the treatment of a patient with Fanconi anemia by cord blood transplantation from a sibling who was born after detailed single cell genetic testing of very early embryos. The pediatric hemato-oncology unit and bone marrow transplantation department at Sheba Medical Center were also among the pioneers in transplantation in patients with advanced CGD and in curing PID by using cord blood transplantation without conditioning.

The field of PID is flourishing in Israel, with many medical centers caring for patients with various forms of PID. In the last 10 years, more than 200 research papers on PID have been published by pediatric immunologist from Israel, and more than 100 hematopoietic stem cell transplantation have been performed. With the implementation of newborn screening for all infants in Israel, severe combined immunodeficiency (SCID) patients have had a successful rate of transplantation [13].

GENE FOUND FOR THE FIRST TIME IN ISRAELI PATIENTS

Israel is a country with diverse populations, some of which have a very high rate of consanguinity. For example, while in the rest of the Western world 70% of patient with CGD have an X-linked inheritance, in Israel the situation is just the opposite and about 70% have an autosomal recessive inheritance. Furthermore, our newborn screening for SCID showed that the incidence of SCID in Israel is 2.5 times higher than in the rest of the world [13]. It is not surprising, therefore, that quite a few of the new genes associated with PID were described for the first time in Israel.

Table 1. Genes that were first found in Israeli patients

Gene	Year of discovery
ATM	1995
FUCTI	2000
FERMT3	2008
ORAI1	2009
LRBA	2012
VPS45	2013
TPPD	2015
STN1	2016
LAT	2016
EFL1	2017
DBR1	2018
ZNF341	2018

ATAXIA TELANGIECTASIA

Ataxia telangiectasia was first described in 1926 by two Czech neurologists: Syllaba and Henner. They described three siblings who presented with chorea atethosis and ocular telangiectasia. Another case was described by a Belgian pediatrician in 1941. In 1958, two American neurologists described an extensive series of patients and provided a detailed account of the disease characteristics, including recurrent pulmonary infections, and named the disorder ataxia telangectasia (A-T).

Several years later, Dr. Robert Good characterized the immunodeficiency associated with A-T, mainly low IgA and IgG with high IgM, absence of antibody response to recall antigens, and impaired T cell response.

Due to the increased interest in A-T, starting in 1980, workshops were conducted in Europe and the United States. A roadmap was designed to resolve key molecular and genetic interactions as it was clear that A-T is a multisystem condition [14].

In the summer of 1977, an Israeli graduate student, Yosef Shiloh, was exploring research options for his doctoral thesis. He had just completed his MSc thesis in human genetics at the Hebrew University–Hadassah Medical Center. His mentor, Prof. Maimon Cohen, invited Shiloh to visit an A-T family. Intrigued by the complexity of the syndrome and its devastating prognosis, Shiloh decided to focus on A-T. This decision was followed by research that Dr. Shiloh has continued to conduct [15].

Under the guidance of Prof. Yechiel Becker, Shiloh investigated the cellular phenotype of A-T and concluded that cells from A-T patients had a special difficulty repairing specific types of DNA double-strand breaks (DSBs). The major question at that time was identifying the culprit gene. Positional cloning was just emerging as a way to identify disease genes. The *ATA* locus was mapped to chromosome 11q22-23 by Gatti and colleagues in 1988, and positional cloning efforts followed in many laboratories. In 1995, Shiloh was the first to show that a single gene was solely responsible for A-T, and he designated it ATM [16]. He subsequently published a series of studies in the ATM protein, concomitantly with other labs. ATM turned out to be a homeostatic protein kinase responsible for the cellular response to DNA DSBs and many other cellular circuits. The immune deficiency in A-T was subsequently explained by a defect in V(D)J and class-switch recombination – a result of the defective handling of DSBs in A-T patients [17].

LEUKOCYTE ADHESION DEFICIENCY II

In the early 1980s, leukocyte adhesion deficiency (LAD) was described as mutations in the beta subunit of the Integrins, which leads to increased susceptibility to infections, impaired wound healing, and marked leukocytosis. Ten years later, a child was admitted to our department at Rambam Medical Center with recurrent infections,

severe psychomotor retardation, dysmorphic features, and marked leukocytosis (> 100,000 mm^3). Furthermore, he was found to have a very rare blood group, Bombay, in which the H antigen is missing on the surface of red cells. This finding and the psychomotor retardation with dysmorphic features were never reported in LAD. Furthermore, integrin (CD18) expression was normal on the leukocyte surface. As this was found only in one patient, we could not determine whether it was a new syndrome. Several months later, Dr. Friedman from Hasharon Hospital contacted us. He had seen a patient with the Bombay blood group. When asked about the features seen in his patient, he confirmed that his patient had exactly the same abnormalities and thus it was clear that we were dealing with a new syndrome, which we called Rambam–Hasharon syndrome [17]. We were able to show that the adhesion and the migration of the leukocytes were defective, but we did not understand the causative molecular defect. At that time the various steps of the adhesion cascade were still unknown. In 1990, the ligand for the selectins, sialyl-Lewis X, was discovered by Paulson. As we thought that this could be defective in our syndrome, we asked Paulson for the monoclonal antibody against the ligand. We found that the sialyl-Lewis X (CD15) was almost completely absent on the surface of our patient's leukocytes.

As both the H antigen and CD15 incorporate fucose into their protein complex, we postulated a general defect in fucose metabolism as the primary defect [16]. After conducting some in vitro and in vivo studies, we showed that the adhesion defect was solely due to the inability of the leukocyte to bind to selectin and has nothing to do with the integrins. Thus, we called this new LAD, LAD II, and the one connected to the integrin defect was referred to as LAD I [18].

We then studied the primary defect in fucose metabolism and found a marked decreased in the activity of GDP-d-mannose-4,6 hydratase, an enzyme essential for fucose production. However, no mutation was found in the cDNA of the enzyme, and it was also present in normal amounts.

The last piece of the puzzle was solved when we used a functional cloning approach and were able to identify the specific GDP fucose transporter, which moved fucose from the cytoplasm into the Golgi apparatus, a place where fucosylation into glycoproteins occurs. We found the mutation in a region that was highly conserve during evolution, which pointed to its importance in the transporter function [18].

The pathological mechanisms causing the mental and growth retardation are still unclear, but answers may become more clear with the use of the induced pluripotent stem cell techniques.

VPS45 DEFICIENCY: CONGENITAL NEUTROPENIA TYPE 5

PIDs illuminate basic mechanisms of immune processes. One critical element of this system, the neutrophil, plays a particularly prominent role in the defense against bacterial and fungal infections. Congenital neutrophil defects can involve certain steps throughout the development, maturation, and function of the neutrophils, including endocytosis. Primary endosomal defects, which are responsible for severe immunodeficiency without associated dysmorphism, were first described in 2013 when Prof. Raz Somech and his group at Sheba Medical Center, together with collaborators from the United States and Germany, investigated five children from a Palestinian family who presented with poor weight gain, hepatosplenomegaly, and severe infection with deep seated abscesses. All of the children had severe neutropenia with less than 200 neutrophils per mm^3. Their adaptive immunity (T and B cell) was found to be normal. The first genetic analysis did not revealed any mutation in all of the genes known to be associated with neutropenia and thus the researchers performed homozygosity mapping using single-nucleotide polymorphism arrays. This procedure was followed by whole exome sequencing, and homozygous mutation was found in the VPS45 gene. In the original article, the researchers described a defective endosomal intracellular protein trafficking due to inherited mutations in the *VPS45* gene [19]. This gene encodes a protein that regulates membrane trafficking through the endosomal system. In the neutrophil, a protein complex containing *VPS45* is responsible to beta-1 integrin recycling between the endocytic compartment and the plasma membrane, a process required for cell motility. Indeed VSP45 protein levels were reduced in the patient's cells. This information underlies a new immunodeficiency syndrome involving impaired neutrophil function and has been designated as congenital neutropenia type 5. The cellular phenotypes were rescued by transfecting mutant fibroblasts with the wild type *VPS45* gene, linking this gene specifically to patient symptoms. Furthermore, a zebrafish model of *VPS45* deficiency, specifically created for this study, exhibited marked paucity of neutrophils, mimicking patient phenotype.

STIM1 DEFICIENCY

Rechavi identified a new PID syndrome associated with autoimmune phenomena and muscular hypotonia. The molecular characterization of the patients led to the identification of a novel mechanism for PID resulting from mutations in stromal interaction molecule 1 (STIM1) in the endoplasmic reticulum that activates ORAI1-CRAC channels, thus crippling calcium entry to T cells and muscle cells resulting in a complex clinical phenotype [20].

PID may provide useful insights and complement experimental approaches to the study of the endosome function in health and diseases.

Acknowledgement

I would like to thank Prof. Shiloh, Prof. Rechavi, and Prof. Somech for their help in preparing this manuscript

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Capsule

Fibrin-targeting immunotherapy protects against neuroinflammation and neurodegeneration

Activation of innate immunity and deposition of blood-derived fibrin in the central nervous system (CNS) occur in autoimmune and neurodegenerative diseases, including multiple sclerosis (MS) and Alzheimer's disease (AD). However, the mechanisms that link disruption of the blood–brain barrier (BBB) to neurodegeneration are poorly understood, and exploration of fibrin as a therapeutic target has been limited by its beneficial clotting functions. **Ryu** et al. studied the generation of monoclonal antibody 5B8, targeted against the cryptic fibrin epitope γ377–395, to selectively inhibit fibrin-induced inflammation and oxidative stress without interfering with clotting. 5B8 suppressed fibrin-induced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation and the expression of proinflammatory genes. In animal models of MS and AD, 5B8 entered the CNS and bound to parenchymal fibrin, and its therapeutic administration reduced the activation of innate immunity and neurodegeneration. Thus, fibrin-targeting immunotherapy inhibited autoimmunity- and amyloid-driven neurotoxicity and might have clinical benefit without globally suppressing innate immunity or interfering with coagulation in diverse neurological diseases.

Nature Immunol 2018; 19: 1212

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Capsule

Sequestration of T cells in bone marrow in the setting of glioblastoma and other intracranial tumors

T cell dysfunction contributes to tumor immune escape in patients with cancer and is particularly severe amidst glioblastoma (GBM). Among other defects, T cell lymphopenia is characteristic, yet often attributed to treatment. **Chongsathidkiet** et al. revealed that even treatment-naïve subjects and mice with GBM can harbor AIDS-level CD4 counts, as well as contracted, T cell–deficient lymphoid organs. Missing naïve T cells are instead found sequestered in large numbers in the bone marrow. This phenomenon characterizes not only GBM but also a variety of other cancers, although only when tumors are introduced into the intracranial compartment. T cell sequestration is accompanied by tumor-imposed loss of S1P1 from the T cell surface and is reversible upon precluding S1P1 internalization. In murine models of GBM, hindering S1P1 internalization and reversing sequestration licenses T cell–activating therapies that were previously ineffective. Sequestration of T cells in bone marrow is therefore a tumor-adaptive mode of T cell dysfunction, whose reversal may constitute a promising immunotherapeutic adjunct.

Nature Med 2018; 24; 1459

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