Primary Immunodeficiency Program at AUBMC

(In collaboration with Children's Hospital in Boston and Four Regional Hospitals in the Middle East):

Studies of Immunological Deficiency Syndromes

Congenital defects in innate or adaptive immunity lead to increased susceptibility to infections caused by a variety of infectious agents. The most common immunodeficiency is IgA deficiency, estimated to affect 1 in 400 individuals, is characterized by recurrent otitis media and sinusitis but generally has a good outcome. Alternatively, more profound immunodeficiency such as severe combined immunodeficiency (SCID) are rapidly fatal in the absence of bone marrow transplantation. In between these two extremes, there is a wide range of immunodeficiency characterized by recurrent moderate to severe infections leading to chronic morbidity and mortality.

At AUBMC, we see the full range of primary immunodeficiency. Patients with recurrent infections or suspected immunodeficiency are frequently referred to our center. These patients come from various parts of Lebanon, Syria, and occasionally Egypt, Iraq, and Jordan. The high rate of consanguinity in Lebanon and these neighboring countries significantly contributes to the high prevalence of primary immunodeficiency. The majority of these patients come from families with poor resources who lack insurance or other means to pay for costly laboratory work-up and frequent admissions.

These patients are evaluated mostly in the Infectious Diseases clinic but some are also evaluated in the Hematology-Oncology clinic. The lack of a program in immunodeficiency, the natural “home” for these patients, has been deleterious in the care and follows up of the majority of these patients. Following initial evaluation and basic immunology tests, the majority of these patients are lost to follow up whether a specific diagnosis was reached or not. Genetic testing is not available locally for known genetically defined immunodeficiency syndromes. When a collaborator in Europe or the USA is identified who is willing to perform genetic testing free of charge, families cannot even afford the cost of shipping the blood samples. Social services occasionally may provide assistance for needy families in this regard. When an immunodeficiency is identified and treatment recommended, e.g. monthly IVIG, the majority of the patients are admitted to other hospitals identified by the Ministry of Public Health where they receive their treatment. In general, these hospitals lack advanced technology and equipment leading to suboptimal care sometimes.

The minority of patients have co-existing hematological problems, e.g. Wiskott-Aldrich or Kostmann syndrome, are followed at the Children's Cancer Center of Lebanon within our medical center.

Basic laboratory work-up for immunodeficiency can be performed at our center.

More specifically, the following tests related to the work-up of the patient with suspected Immunodeficiency are available:

1. Ig quantitation, with IgG subclasses, complement levels.
2. Specific antibody titers for Measles, Mumps, Hepatitis B sAg, Varicella are done locally to assay for specific antibody response to childhood vaccines. Antibodies to Diphtheria and Tetanus toxoids are measured at referral labs in Europe.
3. FACS analysis for CD4, CD8, CD56, etc. is available. Additional testing is available on special request.

In research laboratories, the following tests are done:

1. Neutrophil killing assay (incubation of bacteria with neutrophils and staining with Acridine Orange)
2. Rebuck window for migration/adhesion assays.
3. Cytokine levels by ELISA (IL-2, interferon,TNF, etc.)
4. Proliferation assays (thymidine uptake)
5. Apoptosis assays: Fas, TRAIL, TNF, etc.
6. NF-KB, NFAT, and other gel shift assays
7. Immunostaining for various components of the TNF, FAS, or T cell receptor proteins.
8. Activity of kinases, e.g. PKC
9. Sequencing for specific genes of interest.

Other basic biochemical and molecular biology techniques are routinely performed. The assays done in research labs are only occasionally done on patient material mostly because of lack of IRB approval, lack of funds, and lack of dedicated personnel to pursue them. Additional challenges include the lack of some basic immunologic studies, e.g. lymphocyte proliferation in response to specific antigen or certain CD antigen expression on flow cytometry. Although these studies are technically feasible in our research labs, however the lack of funding to do these studies and concern regarding standardization and reporting to clinicians have hindered their use in the clinical management of patients with suspected immunodeficiency. Also, similar concerns apply to genetic testing, which is technically feasible at our institution where the Research Core Facility recently acquired an automated sequencer.

Over the past decade, our laboratory research has focused on the regulation of apoptosis induced by various stress conditions. We have focused on the regulation of apoptosis induced by Tumor Necrosis Factor

(TNF) alpha and Fas in T-cell lines and the role played by various transducers of the death signal including the sphingolipid ceramide.

We have been able to define the mechanisms of generation of ceramide in response to activation of the TNF receptor such as the activation of neutral sphingomyelinase or *de novo* synthesis from serine and palmitate. We also determined that ceramide generation appears to be downstream of activator caspases that are recruited to the cytoplasmic end of the TNF receptor. In turn, ceramide activates downstream "executioner" caspases to drive apoptosis. Other signaling messengers such as reactive oxygen species were found in our lab to signal apoptosis partially through the indirect regulation of ceramide. We found that the major antioxidant in cells glutathione, which acts as regulator of neutral sphingomyelinase, is depleted by the generation of reactive oxygen species following the ligation of the TNF receptor leading to the activation of sphingomyelinase and the generation of ceramide. In Fas signaling, we determined that the generation of ceramide was primarily by *de novo* synthesis and that this was necessary for cells to undergo apoptosis. In the setting of Fas signaling, we found that ceramide generation also inhibited the activation of protein kinase C alpha and theta both of which are important in T cell receptor signaling, generation of interleukin-2, and the proliferation of T cells.

These studies provide the basis for our interest in primary immunodeficiency as a number of the pathways mentioned above are involved in various immunodeficiency diseases. A strong collaboration with the team at Children's Hospital in Boston will markedly expand our ability to apply our research abilities and interests to clinical cases with suspected immunodeficiency.

Specific Aims of the program:

Two leading international immunologists, Drs. Raif Geha and Luigi Notarangelo at The Children's Hospital in Boston and Harvard Medical School, proposed the formation of a collaborative network joining five hospitals in four Middle Eastern countries (Turkey, UAE, Kuwait, and Lebanon) with Children's Hospital in Boston.

The aims of this network include:

1) Strengthening of the local capabilities in diagnosing and managing patients with primary immunodeficiency
2) Enhancing the exchange of expertise between members of the network
3) Identifying previously unknown immunodeficiencies undoubtedly present in the highly consanguineous middle-eastern population.

Initial funding for this network has been sought from the Dubai Harvard Foundation for Medical Research. By joining this network of medical centers in the Middle East and Children's Hospital in Boston we significantly enhanced our existing medical care to patients with primary immunodeficiencies as well as our research program related to immunology. The Primary Immunodeficiency Program at the AUB-MC consists of a group of interested, dedicated, and well-trained team of faculty and residents. This team includes faculty members trained in Immunology, Infectious Diseases, and Hematology-Oncology.

The program developed a clinical service specialized in the care of patients with suspected or established immunodeficiency with dedicated outpatient and inpatient components. The team discusses difficult cases in regular meetings and establishes a regular online consultation service with members of the Immunology Division at Children's Hospital in Boston as well as our partners in other network medical centers. This program allows us to offer a state-of-the-art approach to the patient with suspected immunodeficiency by establishing hitherto unavailable diagnostic tests with the help and supervision of our colleagues at Harvard, gaining input into clinical care from specialists at Harvard as well as other network members, and significantly upgrading our laboratory research abilities. The leadership of the AUB-MC is highly interested in the success, and the maintenance of this program as is evidenced by supporting letters from the Vice-President for Medical Affairs, Dean of the Faculty of Medicine and the Acting-Chairman of the Department of Pediatrics.

The first step for this program was to develop a database at AUBMC that included known patients with immunodeficiency encountered at AUBMC or by our faculty in consultations outside AUBMC. Initially, we reviewed hospital medical records at AUBMC over the past 20 years in order to determine the type and frequency of immunodeficiencies encountered at AUBMC and identified patients who might benefit from our program.

The review included the following discharge diagnoses: *primary immunodeficiency, unspecified immunodeficiency, combined immunodeficiency, common variable immunodeficiency, hypogammaglobulinemia, hyper IgM syndrome, hyper IgE syndrome (Job's syndrome), chronic granulomatous disease, Wiskott-Aldrich syndrome, Kostmann's syndrome, Ataxia-Telangiectasia syndrome, chronic mucocutaneous candidiasis, Omenn's syndrome, complement deficiency, Chediak-Higashi syndrome, and Leukocyte Adhesion deficiency*. IRB approved reviewing these records and collecting the necessary information. Subsequently, this database became prospective with new patients being enrolled as they are encountered by members of the program. As new patients are enrolled, some may require blood, buccal mucosa, or other tissue studies as part of the work-up for immunodeficiency. Some of these tests are done locally in our research labs whereas others are shipped to Children's Hospital in Boston for more advanced testing.

The tests done in our labs are developed and standardized according to protocols provided by our colleagues at Children's Hospital in Boston. All tissue obtained from patients are preceded by informed consent provided to the patient and the parents. A local informed consent form is used as well as one developed at Children's Hospital in Boston. Both consent forms remain at AUBMC along with the database information obtained in the CRF and are kept under lock in the PI's office. No personal identifying data are transmitted to Children's Hospital in the event that tissue is sent for studies. Patients instead are identified by numbers generated from the database in the PI's lab.

In this regard, it is important to distinguish between standardized immune assays that are established as part of this program and research studies that are a significant component of the work up in some patients. Standardized immune assays include those that were not available at AUBMC and that were established with the help of the Children's Hospital team. These assays are very useful in patient care to help us reach a diagnosis and offer treatment when available and constitute the bulk of the laboratory tests done by our program. These tests are also very helpful in eliminating specific diagnoses in patients with atypical presentations. This group of patients is of particular interest to our program because they may have previously unknown immunodeficiency. This is where the research component of our program enters in order to define the genetic and immunologic defects that underlie the said immunodeficiency.

The Program in Primary Immunodeficiencies at AUB-MC has become a major referral center for patients with suspected immunodeficiencies in Lebanon and neighboring countries. Our current expertise in apoptosis, T cell regulation, and neutrophil function are an asset to the network of medical centers in the Middle East that cooperate to tackle the problem of PID in our region. We also serve as referral centers to our partners in this network when cases with suspected defects in apoptosis, T cell regulation, or neutrophil function are encountered. Our partnership with the leading program in immunodeficiency at Harvard provided us with a major boost in tackling currently well-described immunodeficiencies and discovering new ones that are doubtlessly present in our population.

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