Review

Primary Immunodeficiencies: "New" Disease in an Old Country

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Primary immunodeficiency disorders (PIDs) are rare inborn errors of the immune system. Patients with PIDs are unique models that exemplify the functional and phenotypic consequences of various immune defects underlying infections, autoimmunity, lymphoproliferation, allergy and cancer. Over 150 PID syndromes were characterized in the past 60 years, with an ever growing list of new entities being discovered. Because of their rarity, multi-center collaboration for pooled data analysis and molecular studies is important to gain meaningful insights into the phenotypic and genetic diversities of PIDs. In this article, we summarize our research findings on PIDs in Chinese population in the past 20 years. Close collaboration among various immunology centers, cross-referrals and systematic data analysis constitute the foundation for research on PIDs. Future directions include establishment of a national PID registry, raising awareness of PIDs and securing sufficient resources for patient care and scientific research. *Cellular & Molecular Immunology*. 2009;6(6):397-406.

Key Words: primary immunodeficiency disorders, immunodeficiencies, development, research, China

Introduction

Primary immunodeficiency disorders (PIDs) are often quoted as 'experiments of nature' in humans. Agammaglobulinemia (1), severe combined immunodeficiency disorders (SCID) (2, 3) and chronic granulomatous disease (CGD) (4) were the first few entities of PIDs described in the 1950s. Careful documentation of clinical characteristics, infectious agents and immunological features in patients with various groups of PIDs provided insights into the development and functioning of the T- and B-lymphocyte compartment, phagocytes and complements. The availability of more potent immunological assays facilitated diagnosis and precise characterization of immunophenotypes. With the development in other disciplines such as hematology, immunology, microbiology and basic sciences, many classical PIDs were described in 1950-1960s, including reticular dysgenesis (5), Good's syndrome (6), common variable immunodeficiency (CVID) (7), severe congenital neutropenia (SCN) (8), X-linked hyperIgM syndrome (X-HIM) (9), DiGeorge syndrome (10), and complement deficiencies starting from C2 deficiencies (11) followed by the rest of complement components in the ensuing 2 decades.

Aparting from susceptibility to infections as a result of impaired host defense, patients with PIDs often display features of immune aberrations such as autoimmunity, autoinflammatory syndromes and malignancy. With advances in molecular biology and genetics, the distinctive role of the gene products in respective immune pathways and clinical manifestations can be defined. The Human Genome Project allows rapid progress in unraveling the genetic basis of many types of PIDs within the past decade, and permits diagnosis and heterozygote detection, prenatal diagnosis, delineation of genetic variants and gene therapy (12, 13). To date, more than 120 distinct genes accounting for 150 different forms of PIDs have been identified (14). The acumen of clinicians who are able to recognize and delineate clinical phenotypes, together with a constructive interplay with basic scientists, remains fundamental to the discovery of new disease entities.

Primary immunodeficiency - a sociological perspective

Infection has been historically known to be a major cause of mortality in children. Since late 19th century, the availability of techniques to identify the etiologic agents of infectious diseases, effective antimicrobials and vaccination programs saved millions of young lives. Modernization of the society led to an overall improvement in living standard such as nutrition and sanitation, and improved quality and access to medical care for children reduced their susceptibility to infections secondary to malnutrition and other systemic diseases (15). Such progresses not only led to a substantial reduction of infant and childhood mortality rates, but also brought a distinct group of children with infections occurring at unusual frequencies, of extraordinary severity or caused by unusual organisms to the notice of pediatricians. Pediatric

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immunology emerged in the early 1950s as an organized discipline (13), and has been a rapidly developing clinical subspecialty with spectacular scientific vigor.

Discovery of new PID entities and elucidation of novel immune pathways occurs at an enormous rate, and create lots of excitement within and beyond the field of immunology. However, clinicians in many countries are still confronted by a number of practical issues: under-recognition, limited access to specialized laboratory diagnostic facilities, costly treatments such as long-term replacement therapy or hematopoietic stem cell transplant (HSCT), and lack of sufficient specialist centers dedicated to the care of this group of patients.

Immunology research in China

A detailed account of immunology research in China was given by Cao Xuetao, a distinguished immunologist, in his commentary published in *Nature Immunology*, 2008 (16). In the past decade, there have been significant advances in basic research on cellular and molecular immunology, facilitated by multi-disciplinary integration with genomics, proteomics, systems biology and imaging techniques. The National Natural Science Foundation of China (NSFC) provides funding support for 'Key Program' projects on major scientific and technological issues in immunology, and the overall funding increased 10-fold within a decade. There is high intensity of research activities in areas such as developmental and structural immunology, molecular and cellular mechanisms of immune regulation, infection and immunity, immunotherapy and vaccine development, transplantation and tumor immunology, as well as design and application of immunological techniques.

Primary immunodeficiency disorders in China

The earliest reports of PIDs dated back to the 1960s (17). Most were scattered case reports describing clinical features upon presentation with limited immunological data. Patients were often diagnosed late and many, unfortunately, from autopsy findings. In the early 1980s, PID became recognized as an important branch of clinical immunology. In 1981, a pediatric immunology section was formed under Chinese Pediatric Society of the Chinese Medical Association (18). Clear objectives were laid down, which focused on raising the awareness of pediatricians towards PIDs through seminars and training courses, development of laboratory screening tests for PIDs, establishment of PID network and registry, and providing long-term follow-up and treatment for patients. Regular meetings on immunodeficiency disorders were held to promote recognition of PIDs and experience sharing, and training courses for clinicians and immunology trainees were organized in Chongqing and Shanghai.

At the 4th National Pediatric Immunology Conference in 1995, a draft protocol on screening and diagnostic procedures for PIDs was established (19). At the 5th Conference in 1998, it was agreed that a collaborative network and patient registry for PIDs be established to document the epidemiology of PIDs at a national level, and to facilitate patient referral from regional hospitals to 14 specialist centers for further workup and treatment (20). The progress in clinical diagnostics and research on PIDs in China was summarized and reported annually (21-28). The reported number of PIDs in the Chinese literature significantly increased, and there were a number of large retrospective case series from several specialist centers such as Chongqing Children's Hospital (135 cases from 1993-2007) (29), Shanghai Jiaotong University Xinhua Hospital (93 cases from 1974-2003) (30) and Beijing Children's Hospital (72 cases from 2000-2006) (31). However, clinical information was mostly limited to initial presentations, and data on long-term outcomes and prognosis of patients with PIDs are lacking.

Primary immunodeficiency disorders in Hong Kong

Hong Kong has a population of 7 million, and 3.2% of citizens are below the age of 5 years. In the early 1980s, PIDs were largely unknown to pediatricians in Hong Kong. The development of a specialist service for children with PIDs at The University of Hong Kong could be traced back to 1988. The first patients diagnosed and managed in our unit were those with classical features of PIDs, including CGD, Wiskott-Aldrich syndrome (WAS), X-linked agamma-globulinemia (XLA) and leucocyte adhesion defect (LAD). Referrals from various pediatric units throughout the territory were received, and PIDs became increasingly recognized among local pediatricians. From July 1988 to December 1989, six boys with CGD were diagnosed and formed the first clinical case series of CGD in Chinese (32) with detailed documentation of presenting features, infections, outcomes and phagocytic functions. Subsequently, the clinical and immunological features of various PIDs in our locality were described, including LAD (33), WAS (34), and hypo-gammaglobulinemia (35). The establishment of normal reference range of serum immunoglobulins (36, 37) and lymphocyte subsets of Chinese children, as well as development of functional immunological studies formed an important foundation for clinical immunology and research in Hong Kong. Within the first decade of our service, nearly 100 cases of PIDs were diagnosed in our center (38). This provided important information on the epidemiology of PIDs in China as a whole - based on an estimated minimal incidence rate of 1/8000 in Hong Kong, every year there would be at least 3000 new cases of PIDs in China.

Fifteen years of development: Genetic diagnosis for PIDs in Chinese

The study on molecular defects of patients with X-linked CGD in 1995 marked the beginning of research in PID genetics at The University of Hong Kong. The identification of gp91phox mutations in seven CGD patients (39) was the first published report of genetically confirmed PIDs in Chinese. Unique mutations were described, and provided insights into the structure-functional relationship of the gp91phox protein. Subsequently, mutations in *BTK* and *WASP* genes was described in 11 patients with XLA (40) and 9 patients with WAS (41, 42), respectively. Genetic testing for PIDs, though provided a research basis, was a timely development to meet the needs from an increasing number of

patients diagnosed to have PIDs in Hong Kong (43).

At the 5th National Paediatric Immunology Conference in 1999, a PID collaborative and referral network was established and our unit participated as one of the 14 member centers (20). Since 2001, collaboration was established with Chongqing Children's Hospital, Shanghai Jiaotong University Xinhua Hospital and Children's Hospital of Fudan University in Shanghai, and referrals for genetic testing on various PIDs were received from these major paediatric immunology centers. The number of genetic tests offered in our research laboratory continued to expand throughout the years, and collaborations extended to other hospitals in mainland China (Shenzhen Children's Hospital, Guangdong Provincial People's Hospital, Guangzhou Children's Hospital, Southern Medical University Zhujiang Hospital, Guangdong Provincial Children's Hospital, Nanjing Children's Hospital, West China Second Hospital of Sichuan University and Xiangya Second Hospital of Zhongnan University), Taiwan (National Taiwan University Hospital And National Taiwan University and College of Medicine, Yunlin Branch) as well as other countries in the Asia-Pacific region including Singapore (National University Hospital, KK Children's Hospital and Parkway Medical Centre), Malaysia (Universiti Putra Malaysia, Universiti Kebangsaan Malaysia, Medical Centre, Universiti Sains Malaysia) and Australia (Royal Children's Hospital). Genetic tests were appropriately selected based on the detailed clinical information and immunological data provided by the referring doctors. In 2009, tests for 42 genes were made available (Figure 1). Up to September 2009, molecular analyses were performed for over 500 individuals (Figure 2). Among the 259 patients referred to us, genetic diagnosis of PIDs was confirmed in 164 patients (Figure 3), and 162 of their family members were found to be carriers. Retrospective studies on the clinical, immunological and molecular characteristics on several groups of PIDs were performed, including CGD (44), IL12RB1 deficiency (45), WAS (46) and XLA (47, 48), each being the largest Chinese cohort reported and provided unique information about such PIDs in the Chinese population.

X-linked agammaglobulinemia

X-linked agammaglobulinemia was the most common type of PIDs collected in our cohort. In the past 10 years, 79 patients were referred to us and 66 were found to have *BTK* mutations. To the authors' knowledge, *BTK* mutations were reported in more than 40 Chinese patients from other groups of investigators, including thirty from the cohort described by Wang et al (49) and seven by Zhang et al (50), as well as a few Taiwanese (51, 52). Together with those in our cohort, the total number exceeded a hundred. Only 3 mutations were recurrent, so mutation hotspot was not apparent in our population.

A genotype-phenotype correlation in XLA was suggestive in some recent reports (53, 54). In our study, we classified mutations into severe and mild based on structural and functional consequence by bioinformatics analysis. A significant association between genotype and age of disease onset as well as occurrence of severe infections was identified. Our data supported the existence of genotype-phenotype correlation, though discordant phenotypes were also observed in affected members within the same family. However, the establishment of a definite association remains a challenge. Protein expression, age of diagnosis, and clinical severity were phenotypes commonly represented in genotype-phenotype correlation studies, but the ultimate

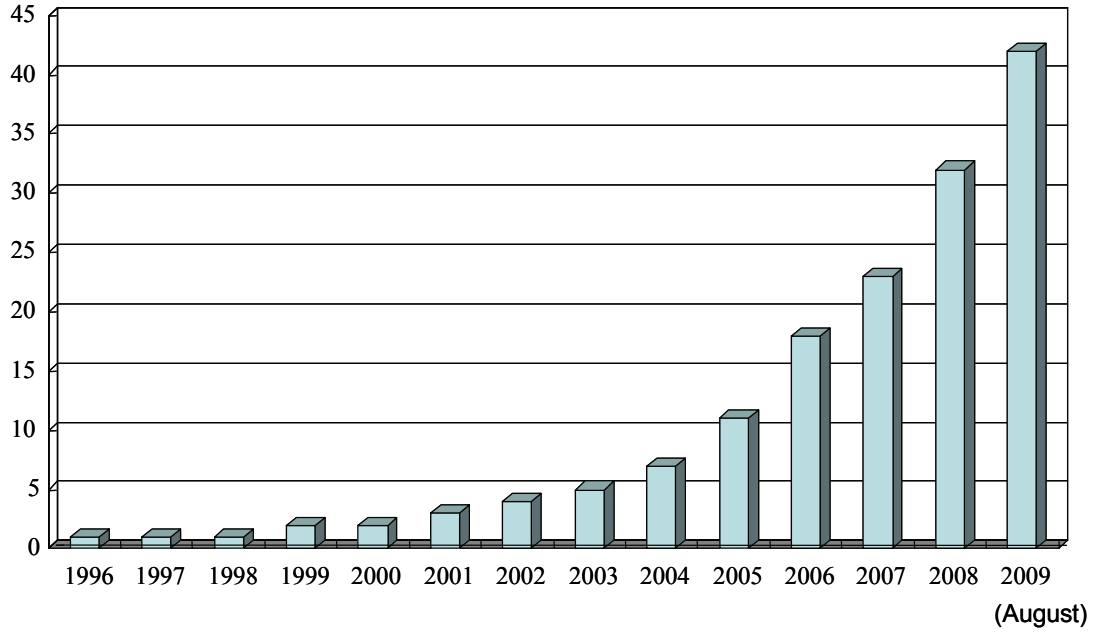

Figure 1. Types of genetic tests for primary immunodeficiency disorders offered at UPAM, in cumulative numbers, from 1996 to 2009.

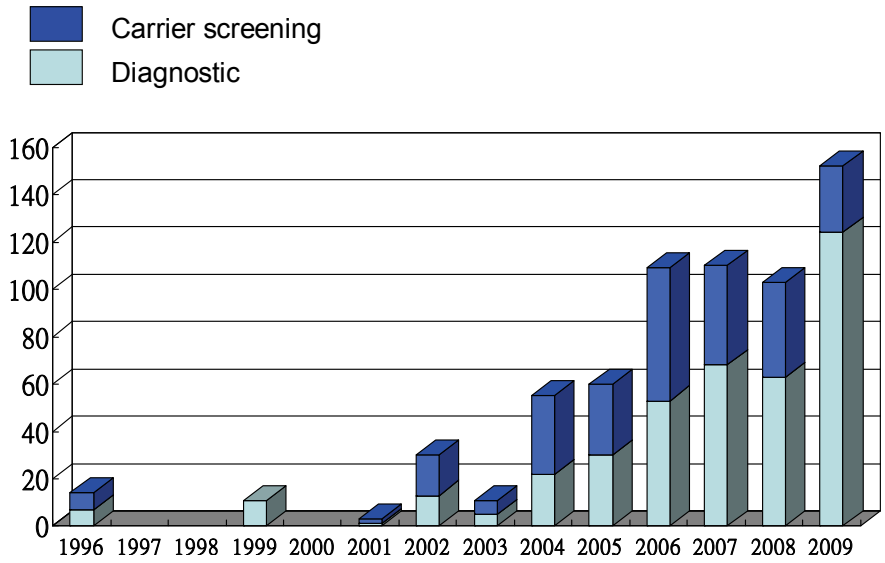

Figure 2. Annual number of genetic tests performed for confirmation of diagnosis and carrier screening, 1996-2009

phenotype of significance should be complication-free, long term survival which is also influenced by modified genes as well as environmental factors. Multi-center collaboration to enable recruitment of a large number of patients with prospective collection of long term follow-up data will facilitate such correlation analysis.

Our cohort, which was of sufficiently large size, allowed us to have a glimpse of the recent development and current issues of PID service in China. The age at onset of symptoms referable to XLA was 2.0 ± 2.0 years (range, 0.1-8.5 years), while the age of diagnosis was 7.1 ± 3.9 years (range, 0.9-16 years). Patients were often treated for recurrent infections, and clinical diagnosis of XLA was made only after multiple, and often lengthy referrals to specialist centers. Over one-third of the patients had male relatives who were known to have XLA or died of recurrent infections in early age, but such family history neither led to a younger age of diagnosis nor initiation of pre-symptomatic immune evaluation. In general, a trend of earlier diagnosis was observed in patients born in the recent decade, reflecting a heightened awareness of PIDs among clinicians in China. However, most of these patients resided in the costal provinces where tertiary medical services were more accessible, and situations in the inland regions were likely to be unfavorable.

HyperIgM syndromes

HyperIgM syndromes are a group of very rare disorders. The incidence of the X-linked form, caused by mutations in the CD40 ligand, is one in a million live births while the autosomal recessive forms are even rarer (55). Since 2004, we identified 15 patients with *CD40LG* mutations. Only 1 patient was diagnosed and managed in our center. In contrast, 6 patients (from 2 Chinese and 2 Indian families) were referred from Singapore and its neighboring countries including Malaysia, Indonesia and India. Because of its rarity, the exact incidence of different forms of HyperIgM syndromes in China and Southeast Asian countries is difficult to determine.

Thirteen different mutations were identified, each being unique to the family. Mutations included frameshift (n = 6), nonsense (n = 3), splice site (n = 2) and gross exon deletions (n = 2) which were all predictive of absent protein production. Missense mutation, which was the most common type of mutation listed in the CD40L base, was not found in our cohort.

Five patients received HSCT, and four of them are currently alive and well. The patient who received mismatch-related donor transplant in Hong Kong suffered from severe chronic gut-versus-host disease and died 5 years post-transplant.

Wiskott-Aldrich syndrome

Over 50 cases of WAS were reported in the Chinese literature; most were isolated case reports or small series lacking outcome data. We identified 35 WAS patients with proven mutations of a wide spectrum distributed across different domains of the *WAS* gene (46), in contrast to a previous report from Taiwan in which majority of mutations involved exon 1 (56). Together with another recent series from Chongqing (57), R41X and R211X were recurrent mutations and both were known to be mutation hotspots. Patients included in the Taiwan and Chongqing cohorts all had classical WAS syndrome. We recently identified a patient with X-linked thrombocytopenia (XLT) phenotype and had L39R mutation. Missense mutations locating in exon 1-4 are more often associated with XLT, which is a milder variant with less severe eczema, immunodeficiency or autoimmunity seldom occur. XLT should be considered as a differential diagnosis in patients with 'chronic idiopathic thrombocytopenic purpura'. Identification of a reduced mean platelet

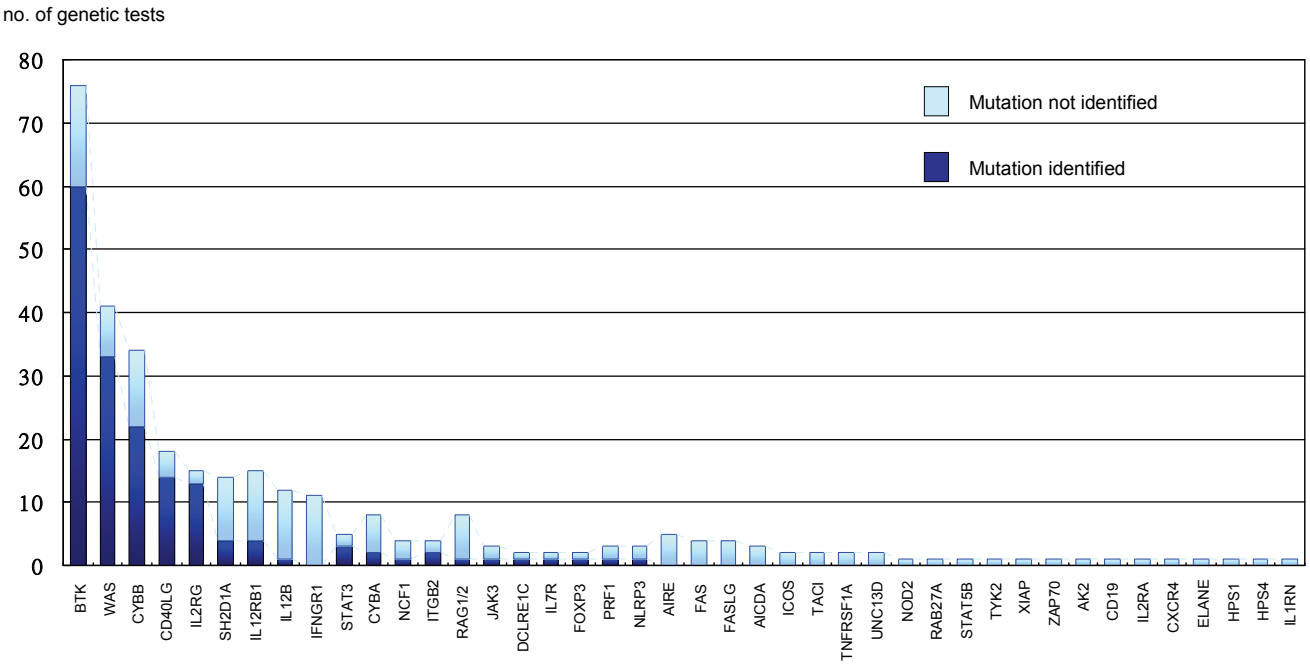

Figure 3. Genetic tests for primary immunodeficiency disorders performed at The University of Hong Kong, 1995-2009. Among 316 genetic tests performed on 259 patients, 164 patients were confirmed to have molecular defects accounting for primary immunodeficiency disorders.

volume is suggestive.

From our series, 7 patients in Hong Kong and 2 in Shanghai received HSCT. All patients are currently alive and well. Successful HSCT in WAS were also reported in Chongqing (58) and Taiwan (56). The reported 5-year survival in the world literature was 70-80%, with a higher success rate among those who received transplant at younger age. Early diagnosis, early initiation of transplant workup and match-unrelated donor (MUD) search is therefore pivotal in the overall prognosis of patients with classical WAS.

Severe combined immunodeficiency disorders

Severe combined immunodeficiency (SCID) is a group of disorders characterized by profound impairment of both T-cell and B-cell immunity. It is regarded as an immunological emergency as patients uniformly die without successful HSCT. Since the initial description of 'essential lymphocytophthisis' by Glanzmann in 1950, more than 10 SCID genotypes are known to date, demonstrating phenotypic, functional and genetic diversity of human SCIDs (14). X-linked SCID caused by the common γ chain (γc) mutation is the most common type, and constituted 44.4% (12/27) of the number of SCID patients referred to us for molecular analysis. We are continuously expanding the diagnostic panel of SCID genes, which include *IL2RG*, *JAK3*, *RAG1/2*, *DCLREIC*, *IL7R*, *AK2* and *ZAP70*.

Chronic granulomatous disease (CGD)

Chronic granulomatous disease is a phagocytic disorder caused by defective NADPH oxidase, leading to absent or severely reduced reactive oxygen species and impaired intracellular killing. The NADPH oxidase enzyme consists of cell membrane–bound gp91-phox and p22-phox and the cytoplasmic proteins p40-phox, p47-phox, p67-phox and RAC2. X-linked CGD, which is the most common form (65%), arises from defect in gp91-phox which is caused by *CYBB* mutations. Autosomal recessive forms are much rarer. Mutations in p47-phox (encoded by *NCF1*) account for about 25%, while p67-phox (encoded by *NCF2*) and p22-phox (encoded by *CYBA*) each constitutes approximately 5% (59). Reported overall incidence of CGD to be 1 in 200,000 live births, the incidence of *NCF1*, *NCF2* and *CYBA* mutations would be less than 1 in a million. In our cohort, *CYBB* mutations were identified in 22 patients. We were able to identify 2 Chinese female patients with homozygous *NCF1* mutation and compound heterozygous *CYBA* mutations, respectively.

From our first cohort of patients with X-linked CGD in Hong Kong (60), we observed that CGD patients had a unique susceptibility to tuberculosis. Hong Kong is a region endemic for tuberculosis, but the incidence of tuberculosis in CGD patients was found to be >170 times higher than that in the general population (17,333 *vs* 100 cases per 100,000 population). We hypothesized that oxidative burst might play a role in host defense against *Mycobacterium tuberculosis*, but the exact mechanism is still uncertain as the evidence from in vitro data in other published studies was controversial. Recently, a comprehensive review by Bustamante et al (61) confirmed a high incidence of tuberculosis infection in CGD patients living in endemic area

such as Iran and Argentina. Our subsequent cohort, which described 17 patients with X-CGD (44), also revealed a high incidence of BCG complications (41.2%), including poor healing of BCG scar, regional adenitis and dissemination. As concluded from Bustamante's review, patients with CGD had high susceptibility to mycobacterial infection, and it is worth to further pursue the relationship between NADPH oxidase and immunity against mycobacteria.

Defects in the interleukin-12/interferon-γ (IL-12/IFNγ) axis

Defects in the IL-12/IFNγ axis constitute a group of rare immunodeficiency syndrome characterized by restricted susceptibility to weakly pathogenic mycobacteria such as *M. bovis BCG*, environmental non-tuberculous mycobacteria and non-typhoid salmonella. To date, 7 genetic disorders were implicated in mycobacterial susceptibility, the most common being *IL12RB1* and *IFNGR1* mutations, each responsible for approximately 40% of the total number of cases, followed by *IL12B* mutations (approximately 10%) while the rest were caused by *STAT1*, *IFNGR2*, *TYK2* and *NEMO* mutations (62). We performed genotyping in 15 individuals with regional, distant or disseminated mycobacterial infection for mutations in *IL12RB1*, *IFNGR1* and *IL12B* genes. Three infants with regional or disseminated BCG were found to have *IL12RB*1 mutations. A teenage girl with disseminated tuberculosis had homozygous mutation -553G>A in the promoter, but expression study was not performed to confirm the functional significance of the mutation. None of the patients had *IFNGR1* mutation.

This was our initial investigation into the occurrence of type 1 cytokine cascade defects in Chinese and Southeast Asian patients. Recently, 4 Chinese patients with genetic defects in the IL-12/IFN-γ pathway were reported in Taiwan. Partial dominant *IFNGR1* mutation (c.818del4) was identified in 3 patients with regional or distant BCG disease, and homozygous missense mutation c.696G>C of the *IL12RB1* gene was identified in 1 patient with prolonged and recurrent salmonella sepsis (63). A search of the Chinese literature published in the past 2 decades revealed that there were at least 20 cases of disseminated BCG in young infants without definite immunological diagnoses. As an increased incidence of TB and BCG complications are also noted in CGD patients, clinicians should be aware of the possibility of primary immunodeficiencies among patients with such infections. We identified several challenges: first, how to initiate timely and appropriate investigations for infants with BCG complications; second, how to identify infants at risk of BCG complications in countries where BCG are routinely given at birth; third, in areas endemic for TB, what is the prevalence of IL-12/IFNγ axis defects among individuals with disseminated TB of unusual severity; and finally, while *in vitro* cytokine production and receptor expression assays are not widely available and discovery of an increasing number of genes involved in type 1 cytokine response is expected, how to design a systematic and cost-effective diagnostic approach towards this heterogeneous disease category. As for investigations, a basic workup with complete blood count, immunoglobulin pattern, lymphocyte subsets and nitroblue tetrazolium (NBT) / dihydrorhodamine (DHR) assays should be performed and HIV infection should be ruled out. Defects in IL-12/IFNγ axis should be considered if the above are all normal. Clinicians should be cautious if the infant has a family history of deaths or adverse events related to BCG vaccination, and BCG should be withheld until immunological workup is completed. The identification of underlying immune defects in individuals with disseminated TB is a challenge in endemic regions. Although adults with underlying IL-12/IFNγ axis defects presenting with disseminated TB have been reported, the occurrence is rare and should probably be suspected when there is relevant family history, recurrent mycobacterial infections or concomitant non-typhoidal salmonellosis. An effective diagnostic approach to pinpoint the molecular defect and arrive at a genetic diagnosis is no easy task in this heterogeneous group of disorders. From our own experience, further studies on other genes may yield diagnosis for those whom *IL12RB1*, *IL12B* or *IFNGR1* mutations were not identified, but is limited by the time, manpower and resource considerations.

The new faces of PID - broadening definitions, novel phenotypes

In the recent decade, there are several major paradigm shifts in the field of primary immunodeficiency on the genotype level, phenotype level, patient and population levels, as elegantly summarized by Jean-Laurent Casanova (64, 65). The classical description of PIDs as rare, familial, monogenic diseases of childhood predisposing to recurrent, opportunistic and fatal infections can only partially depict the concept of 'immunodeficiency'. The complexities of immunodeficiency are recognized by the phenotypic heterogeneity of a particular genetic defect (e.g. *WAS* defect causing Wiskott-Aldrich syndrome, X-linked thrombocytopenia and X-linked neutropenia), genetic heterogeneity of an immunological disorder, defects causing susceptibility to a narrow spectrum or single type of pathogen (e.g. defects of IL-12/IFN-γ axis causing mycobacterial susceptibility, *UNC-93B* and *TLR3* deficiency as genetic etiologies of isolated herpes simplex encephalitis), and adult-onset immunodeficiency such as common variable immunodeficiency (CVID), the genetic basis of which is still largely unknown. Autosomal dominant PIDs without prominent family history are increasingly described, which can arise either from *de novo* germline mutation (e.g. dominant *IFNGR1* deficiency), incomplete penetrance from an affected parents (e.g. dominant *STAT-1* deficiency) or somatic mutations (e.g. dominant *FAS* mutations in the hematopoietic lineage). Clinical heterogeneity is also recognized in some PIDs which are strongly influenced by exposure to specific pathogens and other environmental factors. For example, individuals with defects in IL-12/IFNγ axis may not have any manifestations if they live in countries without routine BCG vaccination policy. It is foreseeable that the definition for 'immuno-deficiency' will broaden, and many genetic alterations underlying immune aberrations will be characterized in the near future.

Table 1. Strategies on future development of clinical service and research on primary immunodeficiency disorders.

Strategies	Future directions
Patient care	Establish more specialist PID centers within reasonable accessibility for all patients in different provinces
	Establish more laboratories which are accreditated for basic immunological evaluation and PID genetic diagnosis
	Formulate up-to-date, evidence-based diagnostic protocols and management guidelines on PID
	Secure government funding or health insurance to ensure that patients are entitled to long-term follow up and appropriate essential treatments, including adequate provision for long-term regular immune replacement therapy or hematopoietic stem cell transplantation
	Establish national PID patient registry for systematic collection of clinical and epidemiological data, with proper dissemination to referring units
Public awareness	Enlist government support, business and charity funding for developing PID clinical service and promoting public awareness
	Provide education and information for the public e.g. website for PID (www.pidchina.org)
Clinician training	Organize annual academic meetings and training courses
	Highlight clinical immunology in undergraduate and postgraduate education curriculum
	Implement formal subspecialty training in clinical immunology for training pediatric and adult immunologists
	Provide continuous medical education courses on clinical immunology / PID to promote awareness among primary care physicians, pediatricians and adult physicians
Research	Strengthen multi-center and cross-discipline collaboration, especially between clinicians and basic scientists
	Promote efficient utilization of immunological techniques and resources for both clinical and research purposes
	Provide opportunities for training future 'clinician-scientists' in the field of PID

Achieving boundless multi-center, cross-discipline collaboration for PID clinical service and research

The collection of a sizeable number of patients with PIDs is an accomplishment made possible by a close collaboration with many renowned pediatric immunology centers in mainland China and other Southeast Asian countries. Supported by research fundings and donations, our unit provides a free genetic diagnostic service to all referring centers, and the use of electronic communications facilitated discussions and referrals. This has partly overcome the physical and resource limitations on PIDs research in our region, and such collaborative efforts provide rich clinical materials for characterizing this group of rare diseases in the Chinese population.

In our cohort, majority of mutations identified belonged to the more common types of PIDs. Of note, genetic defects could not be identified in one-third of the patients with well-defined clinical phenotypes. In particular, molecular defects could not be identified in most patients with suspected immunodysregulatory disorders such as familial hemophagocytic lymphohistiocytosis syndromes, X-linked lymphoproliferative syndrome (XLP), autoimmune lymphoproliferative syndrome (ALPS), autoimmune polyendocrinopathy with candidiasis and ectodermal dysplasia (APECED) and immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX). While we continue to expand the number of PID genes offered in our diagnostic panel, we believe that some patients may have novel PID syndromes whose genetic defects are not yet to be discovered. Definition of the immunological phenotypes by cellular and molecular expression assays is the prerequisite for unraveling these unknown entities. Modern facilities, techniques and expertise of scientists working in basic immunology and molecular genetics in our country will certainly provide a strong armamentarium to make exciting explorations in this arena.

Being the country with the largest population in the world, we potentially have the largest number of patients with PIDs. Ensuring timely diagnosis and optimal treatment in every regional unit is the fundamental issue faced by clinicians dedicated to the care of these patients. On the other hand, systematic analysis of clinical and immunological data by means of a national registry, together with proper collection of cellular and genetic materials will provide immense opportunities for clinical and basic immunology research on these 'experiments of nature'. Immunodeficient patients with underlying genetic defects of the immune system are perfect *in vivo* 'knock-out' models to delineate the complex functions of genes, receptors and signal transduction pathways, as well as immune mechanisms responsible for host defense against pathogens, autoimmunity, lymphoproliferation and development of malignancy. Close collaboration between clinicians and scientists will benefit both medical and scientific communities.

Enlightening suggestions about future directions of PID clinical service and research have been put forward by some distinguished pediatric immunologists in China (66-68), as listed in Table 1. In summary, establishment of specialist PID centers and laboratories, securing adequate funding for regular long-term treatment, provision of training to clinicians and fostering research in immunology are the main

strategies. In particular, we believe that disease surveillance and tracking of long-term outcomes in this group of patients for documentation of disease burden is of great priority. Such information is essential for ongoing needs assessment and performance evaluation. Official inputs from the Government on formulation of policies and resource allocation are needed for sustainable service provision. Initiatives to enhance accountability in terms of improving performance, reducing abuse, compliance with procedures and standards should be implemented. Accountability roles of the Government, Ministry of Health, health care providers, professional associations, researchers, pharmaceutical industry, funding agents and health service users should be defined.

As for research, interactions and communications between clinicians and basic immunologists through different platforms should be maximized, such as study groups, academic meetings and exchange programs. In particular, we need to nurture the next generation of clinician-scientists who are capable of translating discoveries of bench research to solve clinical problems in the ward. It is hoped that the gap between clinical and basic immunological research can be narrowed, and bring about new insights about our immune system as well as revolutions in diagnosis and treatment of PIDs.

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