PROSPECTS OF THE NEW CENTURY

Immunology for 21st Century

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At the turning corner of the century, to reminisce the great achievements in immunology over the past hundred years and foresee its flourish perspectives to benefit human health in the coming century are of enormous values. It is through such activity to pay our tribute to the pioneer immunologists who have created the immunology as the discipline of modern science in biology and medicine; through such activity to thrill the successors make best endeavours to fulfil our contemporary responsibility in the exploitation of frontier immunology and its practical applications.

1 Magnificent achievements of immunology in the 20th century

Taken the advent of chemistry, biology and physics, immunology has been developed as an independent medical and biological science in the mid of this century. The whole immune system was fully illustrated at the level of organs, tissues and cells. The extreme importance of immunological functions in the defence and maintenance of the homeostasis of our body has been soon convincingly demonstrated. It is the immune cells that fight and eliminate invaders from outside environment and from inside generated tumor cells as well as senescence cells to cure infections, reduce the incidence of cancers, and to avoid autoimmune diseases. Application of immunology has blown triumphs in the conquest of numerous human diseases. One of the greatest brilliant victory even since in the human history is the elimination of smallpox by immunological vaccination. The invention of vaccines has enabled human beings to have controlled many devastated epidemic diseases such as plaque, cholera, yellow fever, etc. The poliomyelitis and measles will be the next to be eradicated in the near future. The recognition of antigens by immune cells to produce antibodies resulted in the identification of red blood cell (RBC) types. Transfusion of the blood with matched RBC has been saving millions and millions of human lives. Moreover, the typing of the antigens expressed on lymphocytes has led to the success in organ transplantation with the avoidance of graft rejection.

Immune cells are mobile circulating between lymphoid organs and blood. Readily availability of the immune cells has made them to be the best model to investigate the essential features of cell activities and functions. The convergence of biochemistry, molecular biology and biophysics on the study of immune cells has unveiled the new insight into the fundamental traits of life, such as cell activation signals, signal transduction pathways, and their integrated diverse effects in the activation, or inhibition, of genes for cell proliferation, differentiation, cell machinery products and effector functions, as

well as the activation induced signals for programmed cell death (apoptosis).

An essential and salient discovery is the nature that T and B lymphocytes specifically perceive and respond to the invaders. T and B lymphocytes express receptors (TCR, BCR) which act as antennas to detect and bind to the invader bearing molecules called antigens with the biochemical features distinct from those expressed on lymphocyte themselves. Such receptor recognition of antigen is the very basis that lymphocytes are able to respond to invaders but to be tolerant to the self antigens. It is the core mechanism that modern immunology is founded[1]. The invention of hybridomas and availability of monoclonal antibodies (mAbs) has enabled us to identify and classify immune cells of various types, including antigen-processing cells, antigen-presenting cells, B cells, helper T cells, cytotoxic T cells and suppressor T cells, etc. Thus, immune response is a sophisticated process initiated and regulated by the orchestral activities of a variety sets of immune cells.

A mystery why a cohort of immune cells could act in concordance was not unveiled until mid of 1970s when the T cell growth factors were discovered. Taken the availability of gene cloning technology invented at late 1970s, genes encoding an array of cytokines have been cloned and their recombinant proteins produced. Ample provision for cytokines for research, the fundamental role of these biological proteins has been promptly demonstrated. It is the cytokines that act as sensors and mediators to trigger cell activation events and bring together the activities of various sets of immune cells into harmonious functions; it is the cytokines that function as growth factors and differentiation factors to support immune cell to proliferate and develop into effector cells.

In the process to study immune cell functions, the effector molecules of immune cells were discovered. Though antiserum was experientially used in the late 19th century, it was not until early 1960s that B lymphocytes were found to be the only cells capable of producing antibodies. Later in 1970s to 1980s, the machinery by which cytotoxic T cells (CTL) killing target cells was demonstrated. The cytolysis on the target cells lies on the CTL produced death-inducing cytokines (TNFβ), granzymes and perforins in synergy with the CTL activated process of apoptosis to execute killing of the target cells.

In our lifespan time, all somatic cells constantly die at marked rate. The size of cell repertoire is maintained by the replenishment of nescient cells being given rise from stem cells. It is up-to-date that only the hematopoiesis of immune cells is more clearly revealed than any other cells[2,3]. The discovery of hematopoiesis is one of the milestones in life sciences. It makes possible to identify hematopoietic stem cells (HSC) and study the conditions under which HSC gives rise to all the lineages of blood cells, thereby to continuously generate new immune cells to replenish the cells died after fighting with invaders.

To live and to die are inherently linked two sides of the life. Study of apoptosis has recently appeared as one of the hottest areas in the biological stage. It is notable that most of our present knowledge of programmed cell death (PCD) is derived from T cell-mount death on target cells and from its own death induced by cell activation. Such study opens a new field of cell death biology[4,5]. PCD is a unique process physiologically inherited in the cells. It is initiated by triggering molecules such as FasL and TNF expressed on or secreted by CTL binding to their respective receptors of Fas and TNFR

expressed on target cells. These TNFR family molecules contain death domain in their cytoplasm tails. The death domain binds to the intermediate molecules called adapters which transduce death signals to a series of death executing molecules termed caspase, a family of cysteinyl aspartate-specific proteinases. A cascade reactions of proteolytic activity of various caspases resulted in the cells dismantled. Cells defect in PCD-inducing molecules or resistant to apoptosis will be crucial for the progressing of cancers and autoimmune diseases.

Adaptation of the discoveries in theoretical immunology has opened enormous opportunities for the design of new vaccines and new drugs to be used in prophylaxis and treatment of patients suffered from infections, cancers, autoimmune diseases, cardiovascular diseases, and even other unknown diseases. Immunology has provided tools for organ transplantation and birth control. The immunological products such as recombinant cytokines and chimeric antibodies have been exploited to a new biotechnology industry of powerful pharmaceuticals in clinics and also brought enormous profits to the investors.

For the pivotal discoveries and magnificent contributions to human beings, the Nobel Prize in Medicine has been conferred to the eminent immunologists for 15 times in the 20th century. It is the unambiguous evidence to highlight the excellent contribution of immunology in biomedical science.

2 Immunology striding toward to 21st century

In the 20th century, immunology has developed as the frontier science for uncovering the nature of life and has brought new means to control human diseases. As is the most knowledgeable discipline, immunology today stands at the central stage of modern biology. Immunology is now not only for immunologists but for all the biomedical scientists working together to make life more comprehensive. The immunologists are enthusiastic to untangle the mechanism by which the immune system exerts delicate functions. In essence, we will be embarked to the following fundamentals: antigenic epitopes and TCR/BCR recognition specificity; signals for T/B cell activation, the sorts of integrated signal transduction pathways and the sets of activated target genes, the gene products and the types of functions that T/B cell exert; the fashion of cell-cell communications that affect immune cell functions; T/B cell tolerance to self antigen and factors that determine T/B cell tolerance; factors that break tolerance and antigenic epitope spreading in the elicitation of autoimmune diseases; epitope properties that function as agonists or antagonists for T/B cell activation; T/B cell memory, the unique property that only neural cells and lymphocytes possess; the signals for hematopoiesis that gives rise to the T and B cells; the ways of network regulation that control immune response; and the axis of central neuronal system-endocrine-immune system that harmonizes adequate activities and dynamically maintain homeostasis in whole body. Only by deep understanding of these fundamentals can we fully exploit and utilize the immunological products.

2.1 Dissection of T cell recognition of antigen at structural horizon

Specific immunity is performed by both T and B lymphocytes triggered by foreign antigens. B cells can directly react to antigens without requirement of antigenic processing. B cells recognize an extensive variety of antigens through their expressed B cell receptor (BCR, cell surface bound im-

munoglobulin) to produce antibodies specific against antigens of proteins, sugars, lipid and oligonucleotides. Unlike B cells, T cells can not directly recognize foreign antigens. Rather, T cells respond to the antigens which have been cleaved into peptides and then associated with selfmajor histocompatibility (MHC) molecules. The vast majority of T cells express T cell receptor (TCR) of αβ heterodimer chains. TCRαβ recognizes antigenic peptides anchored in the grooves of MHC molecules and presented by antigen presenting cells. Such property is referred to as MHC restriction of T cell recognition[6]. For that discovery, Zinkernagel and Doherty won Nobel Prize of Medicine in 1996. T cells recognizing antigen through TCR was firstly demonstrated in 1984 when the TCRβ chain encoding gene was cloned[7]. Shortly after that, TCRα gene was also successfully cloned. Amino acid sequence analysis indicates that TCRα and β chains resemble L and H chains of immunoglobulin (Ig). It thrills a hot wave in the hope to understand why TCRαβ recognize antigen in the manner completely different from that of BCR. Various models have been proposed to explain TCRαβ recognition of peptide-MHC coupled molecules based on the amino acid sequences of TCRαβ chains and their antigen-binding site regions, in which the complementarity-determining region (CDR)3 of both αβ chains binds to the middle amino acid residues of peptides, while CDR1 and 2 bind to MHC molecules and, or, the end of the peptide. Even more, all CDRs of 1,2 and 3 might be bound to both peptide and MHC molecule. The precise interpretation was not obtained until the atomic structure image of molecules is recently approached. Determination of molecular structure requires sophisticated technology to produce large numbers of the molecule, process them into crystals and make them with reasonable size and rigid enough to bear the bomb by x-ray. Satisfaction with all these conditions, a crystal molecule will be bombard with x-ray. The differences in x-ray diffraction signals depict the nature of atoms and their precise positions in the molecule. Interactions among the positioned atoms bring the molecule into three-dimensional structure. Analysis of the crystal of a glycosylated TCRαβ bound to peptide-MHC molecule has convincingly manifested. In that, TCR covers the peptide-MHC molecule binding groove where the CDR3s of TCR Vα and Vβ bind to the central position of the peptide, the CDR1 and 2 of Vα bind to the amino terminal region of the bound peptide and MHC molecule, and the CDR 1 and 2 of Vβ bind to the carboxyl-terminal region of the bound peptide and MHC molecule[8]. This molecular atomic image provides the structural framework for the central role of CDR3s of TCR Vα and Vβ in the peptide recognition and the overall TCR recognition of whole peptide-MHC complex.

Among T cells, a minor population of cells express TCR of γδ type . These TCR γδ directly recognizes proteins without requirement of antigen processing, and also respond to non-peptide components. The characteristic of TCR γδ recognition of antigen is fundamentally different from that of TCRαβ, but resembles those of BCR VH and VL. Analysis of the crystal structure of the Vδ domain of a human TCR γδ has revealed that the framework structure of Vδ more closely resembles that of Ig VH than TCRVα. The CDR3 length distribution of TCR Vδ is also similar to that of Ig VH. These crystal study results are consistent with the direct recognition of certain protein antigens by TCR γδ in the similar fashion as that recognised by antibodies. Structural study also revealed that the TCR combining site of Vδ CDR3 is oriented in a similar way to Vα CDR3[9]. The structural features of TCR Vδ with partial similarity to Ig VH and Vα CDR3 may explain the unique antigen recognition of properties of TCR γδ T cells.

Analysis of TCR atomic structure may help understanding the pathogen evasion from immune attack and provide information for drug design. It is known that HIV infection of $CD4^+$ T cells is mediated by HIV envelop protein gp120 binding to its receptor identified as CD4 molecules and its co-receptor of CCR5 (or CXCR4), a member of chemokine receptor family. Recombinant gp120 and soluble CD4 have been developed as candidate vaccines for clinical trial. Both vaccines are turned out to be failure. The antibodies (Abs) elicited by vaccination can only neutralize laboratory adopted HIV isolates, but not neutralize the primary HIV isolates from infected patients. The success in making crystals of the complex of gp120 together with CD4 and neutralizing Ab has provided information of molecular structures and interactions among the epitopes of three molecules[10]. The neutralizing Ab binds to the CD4-binding sites and CCR5-binding sites on gp120 molecules. The amino acid sequences of these receptor-binding sites are relatively conservative. However, they are not exposed in the static gp120 protein, and hence are not accessible to the Ab. During the process of HIV infection, binding of gp120 with CD4 leads to conformational change and exposure of CCR5-binding sites which results in further conformational change to expose the fusion sites of gp41 capable of fusing gp41 to target cell membrane and virus entry into cells. All these changes necessary for virus entry into $CD4^+$ T cells, while the short-time exposure of receptor-binding sites does activate T and B cells and elicit neutralizating Ab production. Based on this observation, a vaccine has been designed and used in immunization of mice by capturing the structure of such transiently exposed CD4- and CCR5-binding sites on gp120 complex with CD4 and CCR5 molecules expressed on cell surface by fixation with formaldehyde. It elicited those Abs that could neutralize the vast majority of primary HIV isolates tested[11]. Thus, the experimental practice derived from atomic structure has gained insight into the protective Ab immune response against HIV infection. The receptor-binding sites of gp120 may be perceived as more effective components to replace the whole gp120 protein for the design of HIV vaccine.

Molecular structure study is, however, hampered by the facility of sophisticated technology and equipment. It is so far not many immunological proteins being crystallized and analyzed at atomic level. Though general patterns of the structures of different molecules have not yet been obtained, the accumulated database of molecular structures of limited proteins attained from x-ray crystallography has provided useful information as references for molecular modelling. The advantage of the technology approaches in many aspects, it is now amenable to predict the secondary structure of the proteins. With the popularity of gene cloning, it makes the enormous number of proteins and their primary amino acid sequences are available, In parallel, monoclonal antibody (mAb), genetically engineered Abs and combinatorial peptide libraries constructed in phage display system provide powerful tool to analyze epitope structure and thus to predict protein conformations by molecular modelling with computer algorithm of the known crystal proteins[12]. The 2D and/or 3D structure of the new proteins acquired from these studies will also be helpful at least in part to drug design.

2.2 T cell recognition of antigen is degenerate

The canonical concept of T cell recognition is that one T cell clone expressing a unique TCR can only recognize one antigenic epitope and elicit a specific response to the stimulated peptide. Recent studies of the role of self-peptides in the selection of T cell repertoire within thymus in MHC defect

mice have revealed the astonishing results. In such mice, one self-peptide-MHC complexed molecule supposed to be recognized by one TCR expressing thymocyte clone could positively select majority of T cell clones (50% of that in wide-type mice) with a broad diverse of TCRαβ specificity. They are able to respond to many antigenic peptides presented by identical MHC molecules. Some of the peptides to which they responded even have no amino acid homology with that peptide for positive selection[13,14]. In line with this, the periphery T cells are highly crossreactive with various different peptides. Such T cell recognition property is termed as degeneracy[15]. The plasticity of T cell recognition of peptides is confirmed by amino acid replacement assay[16] and screening of combinatorial peptide library. An individual T cell clone expressing one specific TCRαβ could respond to a variety of peptides derived from amino acid replacement of the primary peptide presented by same MHC molecule. Thus, one clonotypic TCRαβ may not restrainedly recognize one peptide with fixed amino acid sequence, which may recognize a group of peptides with conformational similarity and with the property that reserves the key anchor amino acid residues at special positions could chemically bind to the respective amino acids on TCR molecule. The T cell recognition degeneracy is a true feature of T cell functions. Mathematical estimation indicates that the actual number of T cell repertoire is much less than the number of peptides presented by MHC molecules[17]. But T cell repertoire shows the potential to respond to countless number of peptides. It is perceivable that T cell recognition degeneracy benefits enormously to our life. With this property T cells can respond to the newly emerged antigenic peptides in the mutated pathogens and are able to protect hosts from new invaders which frequently emerged in the environment. T cell recognition degeneracy is a natural design of evolution to defend against new pathogens in view that the rate of genetic changes, leading to the generation of new TCR or BCR, in mammalians is much slower than that in bacteria and virus which are emerged as new strains of pathogens. Taken HIV as an example, though the virus apparently emerged in late 1950s and the first case of AIDS patient was diagnosed in 1981, all the patients infected with such lately emerged virus mount strong immune response against HIV at the early stage of infection.

Mechanism for T cell recognition degeneracy is not well defined. Part of the reasons comes from TCR recognition property. TCR binds to the complex of peptide-MHC molecules in which CDR1 and CDR2 of TCRαβ bind to the α helix of MHC molecules and hence contribute to T cell recognition. Also, a peptide presented by MHC class I molecule to $CD8^+$ T cells is composed of 8—10 amino acid residues, of which only 2—3 residues are critical for the binding to TCR, the rest residues are replaceable. These factors may contribute to the crossreactivity of TCRs.

The T cell recognition degeneracy may have adverse effect in the elicitation of autoimmune diseases. It may occur when the peptides derived from pathogens possess high affinity to the TCR of autoreactive T cells. Though T cell crossreactivity is an essential feature for T cell response, the occurrence of autoimmune diseases is relatively rare. On the whole, T cell response appears as highly antigen specific. T cell response is predominant to a specific antigen which is preferentially presented by antigen-presenting cells (APC) and bound to the TCR with high affinity. It is probably due to the low concentration, inappropriate binding to MHC molecule, less effective presenting by APC and low affinity binding to the TCR that makes many peptides with some degree of homology to the antigenic peptide either unable to, or only partially activate an individual T cell clone to respond, thereby to

show up as specific response and maintain self-tolerance. Demarcation for the T cell crossreactivity to the foreign antigenic peptides and the avoidance to provoke autoimmune diseases is a subject of important implications[18].

2.3 Connection of signal transduction pathways and cell effector functions

Soon after the discovery of T cell receptor (TCR) genes, immunologists realized that stimulation of TCR by antigen or coated anti-TCR mAb usually leads to T cell anergy. Activation of T cells requires both TCR-CD3 delivered signal generated from antigenic peptide-MHC binding and costimulatory signal(s) derived from the binding of integrin receptors and respective ligands, the pairs playing key costimulation role in the latter are CD28-B7 and CD40-CD40L being expressed on T cells and antigen presenting cells (dendritic cells, macrophages, and B cells). Stimulation by two signals, T cells can be activated to produce cytokines such as IL-2, IL-4, IL-6, IFNγ, etc. These bioactive proteins trigger T cells to proliferate, differentiate and develop into effector cells to execute various T cell functions and eventually lead to T cell apoptosis by activation of programmed cell death (PCD) pathway[19,20]. Analysis of multiple T cell activation signals has illustrated multiple signal transduction pathways[21,22]. The major forms of cell signal transduction pathways can be summarized at present as: protein tyrosine kinase activation pathway; G-protein coupled raf-MAP kinase activation pathway, JAK-STAT pathway (transduce signals derived from cytokines bound to cytokine receptors) and PI-3 kinase pathway. The assembly of these activation pathways leads to the activation of transcriptional factors which bind to the respective domains on DNA regulatory region and thus to activate the genes and their products to mount cell effector functions. In contrary to the positive activation signalling pathways, the activation of cAMP-PKA pathway and protein phosphatase leads to the down-regulation of cell activation, as well as the activation of PCD pathway results in cell death. The scope of opposite signalling pathways determines biochemical homeostasis within cells, the types of cell functions and, as well, the fate of the cells, survival or die.

The signal transduction pathways are of great importance for the transforming of exogenous signals into appropriate cellular response, thereby, cells are adaptable to the outside environment.

Study of signal transduction pathways is one of the hottest areas in immunology. It is not only to disclose the link between outside environment and inside cell machinery, but also in the hope to design new drugs to efficiently enhance the responses which benefit cell normal life, and suppress the response being detrimental to the cells. In essence, cell signalling transduction pathway is a cascade biochemical activity between activators (mostly are enzymes) and substrates. It is however an area of not well defined. The ways how distinct signalling pathways are interacted (cross-talk) and assembled into an integrated signals are unclear. The fine intermediates of substrates composed of the various signal transduction pathways are not all identified. The transcriptional factors which translocate into nucleus for the activation of respective genes await for further investigation. New discoveries in this field are frequently shown up. A new pathway has been found in T cell activation in that TCR-CD3 signal transduces to STAT5 and activate cell proliferation[23]. An immunological synapse has been lately identified through which to integral TCR-CD3 signal and CD28-B7 signal to amplify TCR-CD3 signal[24]. The integration of these costimulation is mediated by reorganization of actin-myosin driven

transport, a lipid membrane raft rich of kinases to directly recruit to the TCR-peptide-MHC contact and therefore amplify early TCR signalling[23]. A molecule termed cAMP-specific phosphodiesterase-7 (PDE7) has been reported this year as the product of integrated signals derived from TCR and CD28 engagements. The induced PDE7 activates IL-2 production and cell proliferation[25]. Obviously, signal transduction pathway is the area having been exploited with rapid path.

2.4 Regulation of immune response and generation of immune memory cells

Immune response is a continuous process with the steps of initiation, amplification, effector function, waning, then, return to static state. Immune response is elicited by the cohort of immune cells with orchestrated activities among innate immune responsive cells such as neutrophils, macrophages, natural killer (NK) cells, and adaptive immune responsive cells composed of antigen presenting cells (APC), T and B lymphocytes. At the initial stage, the innate immune cells are recruited to the place where invading pathogens are located and initiate inflammation to fight against pathogens and present pathogen expressed antigens to T and B cells to activate specific immune response through clonal expansion (proliferation) and differentiation into effector T and B cells specifically against the pathogens expressing the antigens to which T and B cells are triggered. The process is featured by the predominant response of T and B cell clones specific to the pathogen-expressing antigens. Once these pathogens are eliminated, inflammation is dispersed, and T/B cell clonal expansion is suspended. As a consequence, immune response wanes to the close. Thus, the process of immune response displays as the dynamic of balance, imbalance and rebalance of distinct sets of immune cells. In this dynamic, the immune response is raised at appropriate time and controlled at appropriate scope to ensure the dynamic homeostasis of immune system with the capacity of responding to the variety of invaders. Any aberrance that immune cells fail to respond, or respond to the antigen stimulation at either too low or too high level, the original diseases will be exacerbated and the autoimmune diseases caused by hypersensitivity will occur. In general, the process of immune response is the consequence of cascade interactions of various sets of immune cells mediated by activators derived from pathogens and by the cytokines produced by activated immune cells, the means of regulatory machinery, however, are not well elucidated. Apart from the concentration of antigens, it has positive feedback regulation at the initial stage to amplify immune response, which may, in turn, trigger the negative feedback circuit to downregulate immune response at the late stage. Not much knowledge is revealed to understand the opposite but may be intimately linked positive and negative regulatory circuits. Antigenic epitope spreading has recently been found as an important factor for the amplification of immune response[26]. It indicates that the activation of one T or B cell clone by one antigenic epitope, T-B cell interaction and synergetic functions will lead to generation of more antigenic epitopes and activate more T and B cell clones to expand and amplify the response. Antigen epitope spreading may also be the mechanism by which the immune inhibitory means are produced, such as the activation of suppressor T cells, the generation of CTLA-4 protein, a molecule binding to B7 and down-regulate immune response[27], as well as the up-regulation of Fas expression to trigger cell apoptosis. At the late of immune response, another striking feature is the generation of memory immune cells. Once boosted by the antigen to which the immune cells have been primed, the memory cells will promptly raise potent response to be able to rapidly and efficiently eradicate the pathogens

invading again. It is still a puzzle what is biochemical basis for immune cells to acquire memory property and why memory cells able to mount response promptly and powerfully. We know little about the immune regulation and immune memory[28], the dissection to these key issues should be stepped up.

2.5 Hematopoisis of immune cells and man-made blood cell manipulations

The hematopoietic stem cells (HSC) give rise to multiple lineages of blood cells including lymphoid stem cells, which have potential to differentiate into T cells, B cells, natural killer (NK) cells and thymic dendritic cells (T-DC) under appropriate microenvironment. The essential organ for T cell development is thymus, and for B cell development is bone marrow (BM). In mouse BM, a subset of HSC with the phenotype of Thy-1^{lo}Sca-1^{+} $Lin^{-/lo}$Mac-$1^{lo}$$CD4^{lo}$ikaros^{+} may have committed to lymphoid stem cells (LSC)[3]. Within BM, the LSC differentiates into mature B cells, while some migrate into thymus and differentiate into immunocompetent T cells. The focus herein is to discuss the fundamental aspects of T cell development.

Thymus provides unique environment for thymopoiesis and T cell maturation. Defect in thymic epithelial cell development such as nude mutation in mice will abrogate thymopoiesis and block the generation of mature T cells. Similar defect in humans leads to DiGeoge Syndrome. The intact thymic microenvironment is required for the seeded thymocyte progenitors to differentiate into TCR$\alpha\beta^{+}$ and $\gamma\delta^{+}$ functional mature T cells ready to migrate into periphery. T cell development within thymus is proceeded following an ordered program, which includes three-major stages: TCR gene rearrangement and expression of TCR-CD3 complexed molecules on cell surface; TCR$\alpha\beta$-$CD3^{+}$ cells are subject for thymic selection mediated by the interaction of TCR$\alpha\beta$ and selfpeptides-MHC molecules expressed on thymic stromal cells (TSC), through negative selection, 98% of cells died by apoptosis, while through positive selection, less than 2% cells are rescued from apoptosis[29], these survival cells are undergone functional maturation and become immunocompetence to form T cell repertoire before migration to the periphery[30,31].

The MHC restricted recognition of T cells is acquired during thymic selection. TCR recognition of self-peptides presented by self-MHC is indispensable in thymic selection to ensure that positively selected T cells bearing functional TCR and matured to be tolerant to self-peptides. The role of self-peptides in thymic selection is recently unveiled with prominent characteristics. A single peptide/MHC class II complex uniquely expressed in MHC class II defect mice could induce 20%—50% $CD4^{+}$ T cells to be positively selected in comparison with the $CD4^{+}$ T cell pool in wild-type mice[13,32]. The positively selected $CD4^{+}$ T cells exhibit diverse TCR specificity with wide range of Vβ usage; but the repertoire of selected $CD4^{+}$ T cells is incomplete. Also, two third of these positively selected $CD4^{+}$ T cells are responsive to the syngeneic cells expressing full array of selfpeptides/class II of wild type mice. These observations imply that positive selection occurs earlier than negative selection. The positively selected cells are endowed for negative selection, through which further 30%—70% of the cells are deleted. Thus, positive and negative selections may required different peptides[13], and for the generation of mature T cells of full repertoire and self tolerance, a full array of self-peptides/MHC is required.

Haematopoiesis of T cells is one of the core events in immunology, which replenishes the loss of

T cells after fighting with invaders and maintain the physiological functions of whole immune system. Some critical issues in terms of the mechanisms which trigger and regulate the process of T cell development remain largely unknown. Pursuing the following questions is in process. (i) Factors trigger TCR gene rearrangement. In the earliest thymocyte progenitors, TCR genes are in germline state. TCR genes must be rearranged and then encode the production of functional TCR chains. It needs to define the regulatory elements in TCR gene fragments and the transcriptional factors by which TCR gene fragments are activated for rearrangement to occur. (ii) As is known, TCR recognition in thymic selection is degenerate, the mechanism underlying the degenerate nature of TCR recognition is not very well elucidated. A number of models have been proposed to emphasize the affinity, the molecular instruction and their modified ones for the selection. None of them can give clear picture of how thymocytes are positively or negatively selected. The decisive factors in thymic selection must be revealed, can we then understand the generation of T cell repertoire and avoidance of autoimmune diseases. (iii) Is functional maturation a final stage of intrathymic T cell development? It is a matter of argument, but of importance to understand the requirements for thymocytes to mature into functional T cells.

At the time when the mechanisms of all these key issues of T cell development are elucidated the man-made T cells can be amenable.

2.6 Is discrimination of self from nonself an inherent recognition property of T and B cells?

Discrimination of self tissue expressed antigens from foreign organism or endogenous mutated cell expressed antigens is documented as the dogma of immunology. It is this recognition property that T and B cells respond to foreign antigens and kill the pathogens expressing these antigens. It is this recognition property that T and B cells tolerate to self tissue expressed antigens to avoid autoimmune diseases. The capacity to recognize foreign antigens and tolerate to self antigens is thought to be acquired in the period of lymphocyte development, which allows the cells bearing receptors specific for foreign antigens positively selected and the cells bearing receptors recognizing self-antigens deleted. On this dogma that modern immunology is founded. For this contribution, two immunologists won Nobel prize in 1960. However, a lot of evidence indicate that autoreactive T and B cells are present. The network regulations by idiotype-anti-idiotype antibody circuit and autologous mixed T lymphocyte reaction are examples for the existence of self-reactive T and B cells. An extreme example is the autoimmune disease which initiated by autoreactive T and B cell response to immunological attack of self-cells. Obviously, not all selfreactive T and B cells are clonally eliminated during their development. Nevertheless, T and B cells mostly execute specific immune response against foreign antigen-bearing pathogens and are tolerant to self-antigen, even in the presence of autoreactive T and B cells. The discrimination of self from nonself is, to be more precise, the result of immune response rather than at the recognition level. Given that T and B cells can recognize self and nonself antigens, why normally they do not respond to self-antigens and destroy self cells? It may be attributed to the lack of costimulatory signals as described above. But why costimulatory signals can generate in the infection by pathogens. Beyond the 2-signal model for T cell activation[33,34], it may have some other signals we do not know yet. A speculation is raised that there exists "dangerous signal" derived from interactions between innate immune cells and pathogen[35,36], which then activates the expression of costimu-

latory molecules. The nature of dangerous signal needs more deeper study.

2.7 Study of applied immunology should be scaled up at higher level and expanded into more broad areas

The great breakthroughs and approaches in theoretical immunology have opened bright opportunities for prophylaxis and treatment of human diseases with immunological products than ever before. Immune response is virtually involved in all kinds of diseases and thus brings new hopes for the cure of mysterious diseases. A recent example is that the heart disease may be caused by bacterial infection through molecular mimicry[37]. And Alzheimar's disease might be caused by slow virus infection which accelerates neural cell apoptosis.

2.7.1 Vaccination is still the most powerful means to control infectious diseases. Vaccination is the most efficient and cost-effective way to control and eradicate infectious diseases not only in the past, but also in the present and future[38]. HIV infection is spreading at rapid path. Estimation shows that AIDS patients will be increased to 40 millions in the year of 2 000. The reason is due to lack of effective HIV vaccine. Many cancers are also caused by microorganism infection either in direct or indirect ways. Hepatocellular carcinoma is linked to the HBV/HCV infection; Epidemiological survey has shown the close relevance between hilico-bacterial infection and gastric cancer, herpes virus infection and cervix cancer, Epstein-Barr virus infection and Burkitt lymphoma as well as nasopharyngeal carcinoma. Infections in respiratory tract and digestive system have seized millions and millions of children's life. Chronic infections are probably the criminals for autoimmune diseases and numerous mysterious diseases. Invention of vaccines is the most urgent task to all scientists. In order to control and eliminate HIV/AIDS, it must have the effective HIV vaccine. An attempt to this goal has been worked for many years, but almost all are failed because of high frequency of gene mutation. In view that gene mutation in HIV at multiple RNA sites is rare at the beginning of infection. It is the case occurred in chronic infection. A new strategy for the design of recombinant vaccine which are able to induce protective immune response against mutiple antigenic epitopes and which contains genes able to block virus replication at multiple stages would be predictable to be invented in near future.

Application of vaccination is applicable in many fields. It has been used for birth control and, especially in cancer immunological therapy. Since the discovery of tumor specific shared antigens (TSSA) in recent years[39], the antigenic peptides of TSSA have been used for immunotherapy in clinical trial in cancer patients. The primary results are promising. TSSA is a family of antigens which expressed on many types of cancers, but not normal cells except in testis, therefore, TSSA is also termed as CT(cancer-testis) antigens. The CT antigen-encoding genes are not active in normal cells except in testis, mutagenesis in cancerous cells has made these genes activated and their encoded proteins expressed. Immune system will recognize CT antigens as "foreign" to elicit immune response and mount cytolytic activity on tumor cells.

The new approach in vaccinology is to develop DNA vaccine which is cheap, eatable, stable, easy to transport under different geographic areas, and able to raise constant level of immune response in long time. DNA vaccine will be facilitate for global vaccination at the same time period, the condition required for the efficient elimination of epidemic infectious diseases.

2.7.2 Fully Human monoclonal antibody and genetic antibody. Antibody produced in animal sources will soon lose activity in human use because they are foreign proteins and will elicit host immune response to reject them. With the technological availability of gene knock out and gene transfection, a mouse able to produce fully human monoclonal antibodies has been created[41]. It is developed by knocking out mouse Ab encoding genes and reconstituting with human Ab-encoding genes. In these mice, B cells produce Abs with the same property as those produced by human B cells. Because of difficulties in making mice transgenic for all human Ab encoding genes, it needs more efforts to make fully human mAb practical for application aims. Such Ab is much better than humanized Abs, or chimeric Abs, which are derived from mouse source and still preserve 10% of mouse protein with the potential to induce Abs against mouse protein. As the consequence of the formation of Ag-Ab complex, the chimeric Abs will soon be degraded and induce hypersensitivity. Therefore, fully human Abs will have more bright future for therapeutics.

Making hybridoma to produce monoclonal antibody (mAb) is a big step in immunology. The advent of phage display system, construction of Ig genes in phage vector and screening single chain engineered Ab is today a mature technology[42], which will make mass production of Ab feasible, and hence, would replace the hybridoma for its tedious work and unstable nature. Right now, the problem of developing engineered Ab is its low affinity. How to screen out high affinity of engineered Ab is a trouble to be shot.

2.7.3 Antigenic agonists and antagonists. Molecular crystallography and combinatorial phage display have shown that the appropriate amino acid residues at TCR binding motif of peptide presented by MHC molecules are critical for optimal binding to TCR and activation of TCR bearing T cell clone. Replacement of the amino acid residues at TCR binding motif of the peptide will alter the binding to TCR and alter the signals derived from such TCR-ligand complex, resulting in either augmented response or reduced response, even T cell anergy. The peptides leading to enhanced immune response are called agonists, in contrast, the peptides resulting in the inhibition of immune response called antagonists. Therefore, the natural peptides are editable. The tailored peptides should have immense potential for practical use. The agonists are able to be used to enhance immune response in infectious diseases, while the antagonists be used in the treatment of autoimmune diseases and prevention of organ graft rejection.

2.7.4 Recombinant cytokines. Industrial manufacture of human recombinant cytokines such as erythropoietin (EPO) and granulocyte-colony stimulation factor (G-CSF) has brought benefits to the patients and huge profits for the investors. Many cytokines with important biological functions are in the process for manufacturing. Cytokine is the language that mediates cell-cell communication. It is so far a little proportion of cytokine encoding genes have been cloned. There are still enormous numbers of cytokines awaited for cloning and exploitation. It is no doubt a field worthwhile for investment.

2.7.5 Immune cell therapy and potential for man-made organs. There are many immune cells which have been used in clinical treatment for various purposes[43]. The well known examples in clinical application are bone marrow transplantation, blood cell enriching in stem cells, cytokineinduced killer

cells and dendritic cells as adjuvants for loading antigens. Hematopoiesis from multiple stem cells to give rise to different lineages of blood cells especially the neutrophils and monocytes has been largely successful in mice. It brings hopes for man-made blood cells produced in vitro in mass culture system to replace the blood source from human donors. Just a few months ago, human stem cells have been successfully isolated and cultured in vitro. Under appropriate conditions, they can differentiate into neural cells and interestingly to blood cells[44]. Embryostem (ES) cells possess the potential to develop into all kinds of cells. The key issue is to understand the specific signals required for ES cells to develop into the different types of cells. These signals must come from the special cytokines produced by local environment and from cell-cell interactions between stem cells and microenvironment composing stromal cells. There is also the hope to make human tissues and organs with the cultured human stem cells spawned at the scaffold of shaped organ desired and differentiate into the specific cells provided that the specialized signals are deciphered[45]. When the time comes the production of human organs is enable for transplantation, we will no more worry about the heart disease, diabetes, or kidney failure. However, such research would not run faster than overcome the graft rejection in xenogenic organ transplantation[46]. The creation of dolly sheep may provide a more straightforward way to produce human organs by transferring the right human stem cells into sheep blastocysts and let the embryo sheep to develop into the infants with the desired human organs. The technology at present is hurdled by lack of knowledge for the identification and isolation of the stem cells responsible for giving rise to the respective organs. Nevertheless, transfection of human gene(s) encoding functional proteins such as cytokines into sheep blastocyst cells has been successful to produce the precious human proteins in sheep.

3 Conclusion remarks

The mosaic nature of live and death has been tremendously uncovered by immunology. Immune system as the most knowledgeable and understandable entity among all the organ systems has been objectively put at the centre of frontier biology for scrutiny. Evolution is endless in all kinds of life. Immune cells fight against newly emerged pathogens or mutation derived cancer cells will be never stopped. Molecular mimicry between microorganisms and mammalian cells provokes immune response to attack autologous cells and destroy self organs. Thus, immune cells act as Swiss knife to be immensely benefits to the hosts and meanwhile to potentially bring the hosts with detrimental effects. Judicious exploitation of immune system is prospective to invent the most powerful means to conquer a variety of mystery diseases such as Alzheimer's disease and the newly emerged diseases such as AIDS and hence to improve human health.

The expecting hallmark achievements from the study of immune system will soon be extended to the other biological systems, so as to endow us with better understanding of the life and with more efficient means to bring better life of human beings.

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