Science in China Series C: Life Sciences

www.scichina.com
life.scichina.com
www.springerlink.com

Special Topics

Current research status of immunology in the genomic era

LI HaoWen[1], LI JinZhi[1], ZHAO GuoPing[2] & WANG Ying[1,2†]

1 Shanghai Institute of Immunology, Institute of Medical Science, Shanghai Jiaotong University School of Medicine, Shanghai 200025, China;
2 Shanghai-MOST Key Laboratory of Health and Disease Genomics, Chinese National Human Genome Center, Shanghai 201203, China

This review updates the current status of immunology research under the influence of genomics, both conceptually and technologically. It particularly highlights the advantages of employing the high-throughput and large-scale technology, the large genomic database, and bioinformatic power in the immunology research. The fast development in the fields of basic immunology, clinical immunology (tumor and infectious immunology) and vaccine designing is illustrated with respect to the successful usage of genomic strategy. We also speculate the future research directions of immunology in the era of genomics and post-genomics.

genomics, immunology, clinical immunology, vaccine design

Modern immunology is derived from the understanding of immunity that human beings fight against the infectious diseases. The history of immunity may be traced back to ancient China. "Nasal vaccination", which was used to prevent people from smallpox in Ming Dynasty through having them inhale powders made from the skin lesions of patients recovering from the disease, might be the earliest immunity case ever recorded in China. However, the efficacy and safety of the scab powder carrying smallpox virus were not satisfactory. The cowpox vaccine introduced by Edward Jenner was applied against the smallpox at the end of the 18 th century, leading to the eradication of the smallpox worldwide. This is the first time that people manipulate the function of the immune system under controlled conditions against the infectious diseases. Modern immunology thus began to develop[1].

The innovation of science and technology is indispensable for the development of immunology. After being branched from microbiology in the mid 20 th century, modern immunology has achieved great success with advance in the experimental techniques of molecular biology, cell biology and genetics. This has led to apparent improvements in strategies addressing the basic principles in immunology, including the structure and function of MHCs (Major Histocompatibility Complex), TCR (T cell antigen receptor) and BCR (B cell antigen receptor)[2] molecules, molecular mechanisms of immune recognition[3,4] and so on. The significance of intercrossing between immunology and the above- mentioned subjects lies in that these disciplines provide new technical platforms for the development of immunology that becomes the persisting forward momentum for immunology research. This enables immunology to act as one of the forefront research areas of life sciences in the 21st century. The immune system and the research on immune responses in turn become one of the best manifestation models for the above-mentioned disciplines.

The accomplishment of Human Genome Sequencing Project (HGSP) has provided new opportunities for im-

Received October 3, 2008; accepted October 8, 2008
doi: 10.1007/s11427-009-0006-7
†Corresponding author (email: ywang@sibs.ac.cn)
Supported by the National High-Tech Research and Development Program of China (Grant No.2006AA02A252), National Natural Science Foundation of China (Grant No.30771965) and Shanghai Pujiang Program (Grant No.07pj14066).

munological research. The global sense of analyses and assessment of the unprecedented amount of information obtained from HGSP and the follow-up studies has not only promoted the blooming of new ideas and novel strategies in immunology, but also made it possible to utilize them optimally and maximally for diagnosis and treatments of immune system-associated diseases. Accordingly, the concept of immunomics[5] has been raised, namely exploring the mechanisms of immune responses by using combinational high-throughput approaches including genomics, proteomics and bioinformatics, etc. In the present review, we intend to provide ideas for bridging the advance in immunology with the power of genomic approaches and speculate the future research directions of immunology in the era of genomics and post-genomics.

1 The impact of genomics on basic immunology

The inquiry of classical basic immunology covers both the phenotypic and the functional manifestation of immunocytes and immune molecules. Molecules engaged in immune response are mainly characterized as diversity or polymorphism, such as major histocompatibility complex (MHC), cytokines or chemokines in the secreted form, and adhesion molecules in the membrane-exposed form. This, to some extent, may equip the immune system with the pluripotent responses to exterior stimuli. But the diversity or polymorphism of immune molecules makes them more difficult to identify and detect. Feasible and cheap detecting methods derived from genomics research largely fit these studies. One typical example is the determination of polymorphism of Human Leukocyte antigen (HLA) (human MHC molecules) that is called HLA typing. Genes encoding HLA molecules are located on the short arm of chromosome 6 and can be subdivided into three loci. Whereas genes located in MHC Class I and Class II loci encode classic HLA class I (HLA-A, HLA-B and HLA-C) and class II (HLA-DR, DP and DQ) molecules, other genes located in the same regions participate in antigen processing and presentation. A remarkable feature of human MHC genes is the unprecedented and unanticipated extent of their polymorphism ever found in the genome. For some HLA loci, more than 250 alleles have been identified by serological analysis. Molecular sequencing has demonstrated that a single serolocically defined HLA allele may contain multiple variants that differ slightly. Because of their significance in transplantation rejection and disease linkage, an efficient and exact method for HLA typing is required. Both the serological analyses based on complement-dependent cytotoxicity and PCR-based methods like PCR-SSP, PCR-SSO or PCR-RFLP rely on the existing data of HLA typing. Furthermore, the sequencing-based method not only facilitates the HLA typing in a more direct and reliable way, but also makes it possible to detect the new HLA alleles more accurately. Sequencing-based HLA typing thus becomes a pretty substitution for the traditional ones gradually[6].

In addition, novel immune molecules can be identified through searching structural homologues using whole-genome scanning and comparative genomics strategies. Toll-like receptors (TLRs) were identified in this way. In the 1980s, a group of receptor molecules called Toll were found in drosophila, which were demonstrated to play an important role in fighting against fungal infections [7]. Later on, the structural analogues of this molecule in the mammal which have the same anti-inflammatory effect were discovered in the genome database of the mammals named TLRs[8]. Further studies demonstrate that they belong to the family of pattern recognition receptors involved in the innate immune responses. At present, TLRs and the innate immune responses initiated by these molecules have become the hotspots of immunology research.

One of the important characteristics of immune responses is that molecules and cells involved in the immune responses exert their functions in a network form. This makes it possible that when pathogens invade, immunocytes and immune molecules are activated rapidly for the effective elimination of pathogens. Therefore, the conventional explanation on the structure and function of a single molecule cannot precisely interpret the characteristics of such immune responses. A global strategy applied in the genomics research, on the contrary, is inclined to upgrade the basic immunology to a systematic level. One of the central issues in current immunology research is the exploration of molecular and cellular mechanisms of immune responses at different phases. Gene expression profiling analysis is first adapted to elucidate the molecular characteristics of immunocytes or immune molecules during the activation and differentiation. For instance, naïve $CD4^+$ T cells differentiate

into either Th1 or Th2 cells upon different antigen stimulation. These two populations can be characterized as different cytokine secretion patterns: IFNγ secretion by Th1 cells as well as IL-4 /IL-5/IL-13 by Th2 cells. The cytokines secreted by polarized $CD4^+$ T cells mediate further immune responses and terminal effects. But molecular properties of these two populations and mechanisms of the differentiation processes are still mysterious. Results from genome-wide gene expression analysis of early Th1/Th2 differentiation reveal the certain transcription factor network that is important for the terminal differentiation and the expression of functional groups of genes for the biological properties of Th1/Th2 cells. These data to a large extent enrich our understanding of $CD4^+$ T cell differentiation[9,10]. Similar strategy has also been applied to dendritic cells[11] research either in the physiological or pathological status[12,13], which provides not only the phenotypical information but also molecular mechanisms of immune responses DCs engaged under different situations.

2 The impact of genomics on immunopathology

Besides the above-mentioned development of immunology theories driven by genomics research, exploration of pathogenesis that immune system might be involved, the so-called immunopathology, is another important aspect. Unlike traditional pathology that focuses extensively on the nature of diseases and their causes, processes, development and consequences, immunopathology emphasizes more on the relationship between the pathological characteristics and immune responses. Diseases thus become good models to characterize the immune responses under the pathological state, which extends immunology theories as well as provides clues for the diagnosis and treatment of diseases from the perspective of immunology.

(1) The impact of genomics on tumor immunology. Tumor biology and tumor immunology are among the disciplines that benefit much from genomics research. Tumorigenesis is a multistep process including transformation, proliferation, progression, invasion and metastasis. At every step there are certain genes undergoing abnormal or ectopic expression, leading to the alteration of biological behaviors. The proteins encoded by these genes are so-named "tumor antigens" or "tumor biomarkers" in the research area of tumor immunology. While tumor biology emphasizes particularly on the mechanisms of tumorigenesis, tumor immunology tries to survey a variety of tumor antigens that might be recognized by T and B lymphocytes and illustrate the immune responses that might elicit protective immunity to tumors. The achievements of genomics research and the derivative techniques such as genechips, differential display and differential proteomics techniques further facilitate the development of tumor immunology[14].

Immune effectors like CTLs and antibodies against tumors are good mediators for the identification of tumor antigens. MAGE, which is first listed as tumor specific antigen in human, is identified through screening of a cDNA expression library using tumor-specific CTLs established from a tumor patient. The cDNA expression library was constructed by using the tumor specimen from the same patient [15]. New tumor antigen candidates can also be available based on the fact that there exists high-titer IgG specific to spectrum tumor antigens in the serum of tumor patients. These high-titer IgGs can be adapted to screen the homologous cDNA expression library derived from tumor patients[16]. Nevertheless, the primary data from library screening is not evident to demonstrate their tumor-specific or tumor-associated property. Therefore, the association study of candidate molecules with tumors needs to be performed first of all. In addition to using traditional sample-based reverse-transcription PCR or immunohistochemistry assay, the global genome or EST data in public database provides a short cut to determine expression patterns of these candidates. The extensive application of Genechips or tissue chips also accelerates the process of property definition. So far, there are more than 2000 tumor antigens determined through CTLs or auto-antibodies responses. Some of them are demonstrated to be good vaccine candidates undergoing clinical trials. Whereas tumor-specific antigens recognized by CTLs evoke effective CTL responses against tumors, the clinical significance of auto-antibodies against tumor antigens is still under investigation, such as the relation between auto-antibodies and pathological behaviors including classification, stages, early diagnosis, recurrence, metastasis, and mortality rate etc. Once there comes the breakthrough of issues mentioned above, these tumor-specific auto-antibodies will facilitate the diagnosis, monitoring and treatment of tumors through intercrossing with genomics technology such as antibody chips technology.

(2) The impact of genomics on infectious immunology. The concept of immunity to infection refers to a series of immune responses triggered by the immune system to defend the invasive pathogens. The outcome of immune responses thus relies on both pathogens and hosts: If pathogens are able to escape from the attack of the immune system, the hosts suffer from tissue injury even lethality. If the immune system can provoke efficient immune responses, the infection can be controlled and the pathogen be eliminated soon. To explain the immunological mechanisms involved in these contrary outcomes, genomics approaches have been introduced.

Genomics has a deep impact on both sides due to the interaction between pathogens and hosts. From the side of pathogens, virulence factors and possible pathogenic mechanisms can be deduced according to genome sequences and pathogenic effects through comparative genomics. From the side of hosts, one of the important inquiries associated with genomics is the study on genetic susceptibility/resistance to infection by detecting single nucleotide polymorphisms (SNPs) of immune-related molecules. The intact human genome consists of 3 billion nucleotide basepairs, of which 99.9% share similarity at the individual level. Variations occur among the resting 0.1% in which 85% are SNPs. SNPs thus represent the most common hereditable genetic variation in human beings. With the advantage of genomic techniques and public bioinformatics databases, it is accessible to study the SNPs with great convenience. On one hand, re-sequencing technique has unmasked the SNPs in the association study in a more precise way through combining with epidemiological study. On the other hand, more programs have been designed and released for mapping the SNPs haplotype blocks, Tag SNP selection and SNP linkage deequilibrium studies, which motivates the association study of SNPs with infectious diseases. Several reports have described the recent advance in SNPs analysis in association studies. For instance, interferon and its downstream signal transduction are dedicated largely to anti-HCV infection immunity. It has been reported that SNP mutations at four sites of the interferon regulatory factor 1(IRF-1) gene are closely associated with HCV infection[17]. Similarly, SNPs in IL-1 gene loci have been linked with the helicobacter pylori infection[18], while SNPs in TLR genes are associated with sepsis[19]. Although the present association studies are more focused on the characteristics of SNP mutation patterns, with the development of functional genomics disease-associated SNP studies will shift from phenotypic analysis to functional prediction, including SNPs and the binding capacity of a given gene to transcription factors, SNPs and structure and biological function of immune molecules, SNP mutations and the outcomes of immune responses, etc. These studies will provide direct evidence for the potential of SNPs in prevention, prediction and treatment of infectious diseases.

3 The impact of genomics on vaccine designing for immunotherapy

One of the ultimate goals of immunology research is to discover vaccines which can arouse specific, strong and sustained immune responses for prevention and treatment of clinical diseases. Consistent with the goals of immunology, genomics not only illustrates the basic principles of life science, but also acts in the battle against the diseases. The global genome data and bioinformatic approaches are playing more and more significant roles in vaccine designing.

There are three generations of vaccines, that is, live attenuated whole pathogens, protein subunits, and epitope-based molecular vaccines (including peptide vaccines and nucleic acid vaccines). One of the most commonly used approaches in vaccine designing is to determine the appropriate vaccine candidates starting from scanning the whole genome sequences, which is also called "reverse vaccinology"[20]. Unlike conventional approaches starting from the large scale cultivation of pathogens and purification of proteins from pathogens, reverse vaccinology relies more on the existing genome database to predict the candidate antigens that fit the gold criteria of a potential vaccine through combinational algorithms. The emerging whole genome database of pathogens fulfills the request for vaccine designing in reverse vaccinology. First of all, open reading frames (ORFs) are deduced along the pathogenic genome by using professional ORF finding software. Then subcellular localization of putative proteins deduced from ORFs is analyzed. Those with the secreted or surface-exposed properties and less or no homology to the human genome sequence are more suitable as vaccine candidates for further biological evaluation. By using reverse vaccinology, vaccine designers can greatly minimize the number of vaccine candidates for verification which makes the entire process less time- and

money-consuming.

One of the pioneer examples of reverse vaccinology is the investigation of vaccines against *Neisseria meningtidis* which is the main pathogen for sepsis and meningococcal meningitis. Through scanning the genes encoding capsular capsule commonly expressed in five serotypic stains and manifesting a series of *in vitro* and *in vitro* experiments, seven capsular proteins immunogenic to a broad range of strains are identified for further clinical evaluation[21,22]. Similar research strategy has been applied to the study of vaccine designing against *Staphylococcus aureus*[23,24] and pseudomonas aeruginosa[25]. In China, reverse vaccinology has also been initiated for vaccine designing. Based on the whole gene sequencing of *L. interrogans* serovar Lai strain accomplished by Ren and his colleagues[26], 4727 open reading frames were deduced. By using bioinformatics, comparative genomics hybridization and transcriptome analysis, Yang and his co-researchers further have predicted 616 genes encoding surface-exposed proteins, of which 226 genes are considered as potential vaccine candidates after determining their conservation of distribution in certain epidemic serovars and high expression in given serovars[27].

Following the determination of vaccine antigen candidates, the next step is epitope-mapping of these antigens that can arouse efficient T cell and B cell immune responses. There are several common properties of T cell epitopes. First, T cells recognize an antigen in the form of continuous peptides presented by MHC molecules after being processed by antigen presenting cells. Secondly, the peptides bound to MHC class I molecules with a closed groove are generally nanopeptides while MHC class II molecules bind to a peptide of 12—20 amino acid residues in length due to the open end of the grooves. The two types of peptides interact with MHC molecules with anchor residues at relatively fixed sites. According to the shared structural property and experimentally determined affinity data of MHC binding peptides, professional software has been edited for the prediction of T cell epitopes and their binding capacity to MHC molecules, such as BIMAS[28], ProPred[29], SVMHC[30] and MHCpred. The predicted T cell epitopes then undergo *in vitro* and *in vivo* experiments to verify their capability of provoking antigen-specific immune responses. Such prediction has already been reported in designing of vaccines to tumor antigens like MAGE1[31], HER-2/neu[32] and OVA66[33], as listed in Table 1. Besides, according to the basic principle of MHC binding, some amino acid residues of T cell epitopes can be further replaced or modified with the help of software like QSAR[34]. Such peptides which are called "supermotifs" may possess higher binding affinity with similar or stronger capacity to induce immune responses, which play important roles in the vaccine designing against tumors.

Table 1 List of T cell epitope encoded by tumor antigen genes

Genes	MHC class I-restricted		MHC class II- restricted	
	HLA allele	Amino acid sequence	HLA allele	Amino acid sequence
MAGE-A1	A1	EADPTGHSY	DRB1*1301	LLKYRAREPVTKAE
	A3	SLFRAVITK		
	A24	NYKHCFPEI		
	B37	REPVTKAEML		
	Cw2	SAFPTTINF		
NY-ESO-1	A2	SLLMWITQC	DRB4*0101	VLLKEFTVSG
	B*3501	MPFATPMEA		
Gp100	A2	KTWGQYWQV	DRB1*0401	WNRQLYPEWTEAQRLD
	A2	AMLGTHTMEV	DRB1*0701	TGRAMLGTHTMEVTVYH
	A3	LIYRRRLMK		
	A24	VYFFLPDHL		
	B*3501	VPLDCVLYRY		
	Cw8	SNDGPTLI		
Melan-A	A2	AAGIGILTV	DRB1*0401	RNGYRALMDKSLHVGTQCALTRR
	B45	AEEAAGIGIL		
HER-2/neu	A2	KIFGSLAFL	DR11	GSYVSRLLGIGLVPIKWMALESILRRRF
SURVIVIN	A2	ELTLGEFLKL		

B cell epitope is defined as a precise surface region of an antigen capable of binding to the variable domain of an antibody. The production of pathogen-specific antibodies can boost host immunity in the resistance to the infection mediated by virus, intracellular or extracellular bacteria. Unlike T cell epitopes, B cell epitopes are either linear (continuous) or conformational (discontinuous), of which amino acid residues are not in a sequence but become spatially juxtaposed in the folded protein. So when B cell epitopes are predicted with bioinformatic methods, the hydrophilic or hydrophobic characteristic of the amino acid residues constituting the intact antigen molecule is one of the most important criteria for consideration. Besides, the three-dimensional structures (either X-ray cystallograph or computatively deduced) of given antigens are also advisory for the accuracy and comprehensibility in the prediction of conformational epitopes. Due to the hypervariability of the antigen-binding region in antibodies (or BCR), algorithm-based prediction of B cell epitopes has lagged far behind that of T cell epitopes. Nevertheless, there are some programs available for B cell epitope prediction, including Bcepred, Discotope[35], Bcipep[36]. Another strategy to determine B cell epitopes is to screen the peptide phage display library by using antigen-specific mouse monoclonal antibodies generated by the conventional mouse hybridoma technique. With confirmed neutralization activity of these monoclonal antibodies against pathogens, B cell epitope peptides identified are probably able to trigger effective humoral immune responses, which has been demonstrated in the case of helicobacter pylori[37] and SARS virus surface proteins[38].

4 Perspective

With the development of genomics, all the innovations of its research techniques and strategies have had far-reaching influences on immunology and will play a more important role in the future. High-throughput and large-scale analyses of genome, functional genome, transcriptome, proteome and bioinformatics will continue supporting the intensive development of basic immunology, especially the inquiry of cytokine regulatory network undergoing complex immune responses, the molecular mechanisms of the signal transduction network involved in the maturation and activation of immunocytes, the exploration of the remodeling of the immune responses and so on. Meanwhile, the marriage of immunology with genomics will partly overcome the laboriousness in the process of vaccine design and avoid the leaking of candidate proteins during the conventional experimentation. The techniques presented here probably will accelerate the development of vaccines for the treatment of tumors and infectious diseases like tuberculosis and viral hepatitis.

1 Zhou G Y. Immunology. Shanghai: Shanghai Science & Technology Press. 2007.
2 Mak T W. The T cell antigen receptor: "The Hunting of the Snark". Eur J Immunol, 2007, 37 (Suppl 1): S83—S93
3 Nicholson L B, Kuchroo V K. T cell recognition of self and altered self antigens. Crit Rev Immunol, 1997, 17(5-6): 449—462
4 Takeda K, Akira S. Roles of Toll-like receptors in innate immune responses. Genes Cells, 2001, 6(9): 733—742
5 Klysik J. Concept of immunomics: a new frontier in the battle for gene function? Acta Biotheor, 2001, 49(3): 191—202
6 Santamaria P, Lindstrom A L, Boyce-Jacino M T, et al. HLA class I sequence-based typing. Hum Immunol, 1993, 37(1): 39—50
7 Lemaitre B, Nicolas E, Michaut L, et al. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in *Drosophila adults*. Cell, 1996, 86(6): 973—983
8 Rock F L, Hardiman G, Timans J C, et al. A family of human receptors structurally related to *Drosophila* Toll. Proc Natl Acad Sci USA, 1998, 95(2): 588—593
9 Lu B, Zagouras P, Fischer J E, et al. Kinetic analysis of genomewide gene expression reveals molecule circuitries that control T cell activation and Th1/2 differentiation. Proc Natl Acad Sci USA, 2004, 101(9): 3023—3028
10 Lund R J, Löytömäki M, Naumanen T, et al. Genome-wide identification of novel genes involved in early Th1 and Th2 cell differentiation. J Immunol, 2007, 178(6): 3648—3660
11 Santegoets S J, Gibbs S, Kroeze K, et al. Transcriptional profiling of human skin-resident Langerhans cells and CD1a$^+$ dermal dendritic cells: differential activation states suggest distinct functions. J Leukoc Biol, 2008, 84(1): 143—151
12 Harenberg A, Guillaume F, Ryan E J, et al. Gene profiling analysis of ALVAC infected human monocyte derived dendritic cells. Vaccine, 2008, 26(39): 5004—5013
13 Tarama R, Kato H, Ishikawa Y, et al. Gene expression changes induced by type IV allergy-inducible chemicals in dendritic cells. J Vet Med Sci, 2008, 70(7): 673—680
14 Miles A K, Matharoo-Ball B, Li G, et al. The identification of human tumour antigens: Current status and future developments. Cancer Immunol Immunother, 2006, 55(8): 996—1003
15 Van der Bruggen P, Traversari C, Chomez P, et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science, 1991, 254(5038): 1643—1647
16 Sahin U, Türeci O, Schmitt H, et al. Human neoplasms elicit multiple specific immune responses in the autologous host. Proc Natl Acad Sci USA, 1995, 92(25): 11810—11813

17 Fortunato G, Calcagno G, Bresciamorra V, et al. Multiple sclerosis and hepatitis C virus infection are associated with single nucleotide polymorphisms in interferon pathway genes. J Interferon Cytokine Res, 2008, 28(3): 141—152
18 Liou J M, Lin J T, Wang H P, et al. IL-1B-511 C→T polymorphism is associated with increased host susceptibility to *Helicobacter pylori* infection in Chinese. Helicobacter, 2007, 12(2): 142—149
19 Wurfel M M, Gordon A C, Holden T D, et al. Toll-like receptor 1 polymorphisms affect innate immune responses and outcomes in sepsis. Am J Respir Crit Care Med, 2008, 178(7): 710—720
20 Masignani V, Rappuoli R, Pizza M. Reverse vaccinology: a genome-based approach for vaccine development. Expert Opin Biol Ther, 2002, 2(8): 895—905
21 Pizza M, Scarlato V, Masignani V, et al. Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. Science, 2000, 287(5459): 1816—1820
22 Tettelin H, Saunders N J, Heidelberg J, et al. Complete genome sequence of *Neisseria meningitidis* serogroup B strain MC58. Science, 2000, 287(5459): 1809—1815
23 Wizemann T M, Heinrichs J H, Adamou J E, et al. Use of a whole genome approach to identify vaccine molecules affording protection against *Streptococcus pneumoniae* infection. Infect Immun, 2001, 69(3): 1593—1598
24 Maione D, Margarit I, Rinaudo C D, et al. Identification of a universal group B streptococcus vaccine by multiple genome screen. Science, 2005, 309(5731): 148—150
25 Bodilis J, Barray S. Molecular evolution of the major outer-membrane protein gene (oprF) of *Pseudomonas*. Microbiology, 2006, 152(Pt 4): 1075—1088
26 Ren S X, Fu G, Jiang X G, et al. Unique physiological and pathogenic features of *Leptospira interrogans* revealed by whole-genome sequencing. Nature, 2003, 422(6934): 888—893
27 Yang H L, Zhu Y Z, Qin J H, et al. In silico and microarray-based genomic approaches to identifying potential vaccine candidates against *Leptospira interrogans*. BMC Genomics, 2006, 7: 293
28 Hassainya Y, Garcia-Pons F, Kratzer R, et al. Identification of naturally processed HLA-A2-restricted proinsulin epitopes by reverse immunology. Diabetes, 2005, 54(7): 2053—2059
29 Singh H, Raghava G P. ProPred: prediction of HLA-DR binding sites. Bioinformatics, 2001, 17(12): 1236—1237
30 Donnes P, Elofsson A. Prediction of MHC class I binding peptides, using SVMHC. BMC Bioinformatics, 2002, 3: 25
31 Akiyama Y, Maruyama K, Nara N, et al. Cytotoxic T cell induction against human malignant melanoma cells using HLA-A24-restricted melanoma peptide cocktail. Anticancer Res, 2004, 24(2B): 571—577
32 Correa I, Plunkett T. Update on HER-2 as a target for cancer therapy: HER2/neu peptides as tumour vaccines for T cell recognition. Breast Cancer Res, 2001, 3(6): 399—403
33 Jin S, Wang Y, Zhang Y, et al. Humoral immune responses against tumor-associated antigen OVA66 originally defined by serological analysis of recombinant cDNA expression libraries and its potentiality in cellular immunity. Cancer Sci, 2008, 99(8): 1670—1678
34 Doytchinova I A, Guan P, Flower D R. Quantitative structure-activity relationships and the prediction of MHC supermotifs. Methods, 2004, 34(4): 444—453
35 Haste Andersen P, Nielsen M, Lund O. Prediction of residues in discontinuous B-cell epitopes using protein 3D structures. Protein Sci, 2006, 15(11): 2558—2567
36 Saha S, Bhasin M, Raghava G P. Bcipep: a database of B-cell epitopes. BMC Genomics, 2005, 6(1): 79
37 Reiche N, Jung A, Brabletz T, et al. Generation and characterization of human monoclonal scFv antibodies against *Helicobacter pylori* antigens. Infect Immun, 2002, 70(8): 4158—4164
38 Duan J, Yan X, Guo X, et al. A human SARS-CoV neutralizing antibody against epitope on S2 protein. Biochem Biophys Res Commun, 2005, 333(1): 186—193