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Establishment and characterization of T cell clone induced experimental autoimmune glomerulonephritis in BN rats

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To isolate and characterize the possibly existing autoreactive T cells that induce experimental autoimmune glomerulonephritis in Brown-Norway rats. Methods: Based on the establishment of experimental autoimmune glomerulonephritis model induced by mercuric chloride, single cell suspensions were prepared from spleens of mercury-injected animals, T lymphocyte populations were enriched by nylon wool chromatography, and stimulated with specific autoantigen (laminin) or Con A, then cultured in IL-2-containing RPMI 1640 medium. So the activated and proliferated T cell clone was harvested. Then a series of characters of clonal T cells, including the antigenicity, pathogenic and immunological characters were detected. Results: (1)The ART clone cells were transferred into the normal BN rats, result showed normal BN rats present the same clinical, pathological alterations as the mercuric induced model rats. (2) The proliferation of ART clone was detected significantly activity induced by laminin with MTT. (3) The most of the T cell clone expressed CD3+/CD4+ molecule immune phenotype when detected by flow cytometry. (4) The secretion of cytokines of the T cell clone were measured by ELISA, we found interleukin-4, not the IFN-r, was the major cytokine in the culture supernatant of T cell clone, with a significantly difference compared to that of T cells isolated from normal BN rats. Conclusion: These results showed that autoimmune T cells may be existed in the autoimmune glomerulonephritis induced by mercuric chloride, and the T cell clone had significantly antigen-induced proliferation activity and pathogenesis. The major immune response was Th2 immune reaction. In summary, we thought autoimmune T cell play an important role in the pathogenesis of autoimmune glomerulonephritis than previously realized.

***Keywords*:** autoreactive T cell; experimental autoimmune glomerulonephritis; T cell clone

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Study on immune function of polysaccharides from *Asparagus officinalis*

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The immune function of polysaccharides from *Asparagus officinalis* on S180 tumor mice was studied. After oral administration of polysaccharides solution (25, 50, 100 mg/kg) to S180 mice for a week, thymus and spleen index, anti- sheep red blood cell (SRBC), number of antibody secreting cell (NASC) in spleen and phagocytic activity were detected, and lymphocytic transformation rate (LTR) in spleen was also determined using MTT methods. The results showed that the thymus and spleen index, LTR, anti-SRBC and NASC in spleen significantly increased after administration (3.53±0.80 vs 5.10±0.47 mg/g, $P<0.05$; 5.69±0.92 vs 7.49±1.18 mg/g, $P<0.05$; 1.047±0.012 vs 1.154±0.016, $P<0.05$; 6.46±0.12 vs 8.18±0.29, $P<0.05$; 0.403±0.008 vs 0.471±0.007, $P<0.05$). Phagocytic activity also increased significantly (phagocytic index: 0.53±0.017 vs 0.72±0.029, $P<0.01$); (phagocytic ratio: 32.30±1.098 vs 60.53±2.022, $P<0.01$). In conclusion, polysaccharides from *Asparagus officinalis* enhanced immune function of S180 mice.

***Keywords*:** *Asparagus officinalis* L.; polysaccharides; immune function

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Zwitterionic polysaccharide compared to other derivates of polysaccharides in activation of immune response

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As plain polysaccharide (PS) vaccines do not generate a memory immune response against the pathogen and are not effective in young children, it is necessary to develop nevol strategy to enhance the immunogenicity of PS. Though Zwitterionic polysaccharide (ZPS) could activate T cells through MHCII presentation offers significant opportunities for the design of new classes of vaccines against a variety of infectious diseases, there are relatively few bacterial

polysaccharides that possess a zwitterionic charge motif. In this study we used a chemically modified ZPS that was from a negative polysaccharide, chitosan, to examine the influence of the chemical modified ZPS on immune response. Compared with primary immunization, an affinity maturation process to PS occurred in all the mice after secondary immunization with ZPS. In this study we also compared the immunogenicity of ZPS with a polysaccharide-carrier protein conjugate in mice, a well-established method to improve immunogenicity of PS. The results that PS antiserum and conjugate antiserum could strongly react with ZPS and the ZPS antiserum also did good cross reaction with polysaccharide showed that chemical derivated ZPS is a good potential candidate for development of vaccine. The proliferation of splenic cells crossing-stimulated with different antigens *in vitro* showed that ZPS is better than that conjugate. We observed that ZPS could up regulate the expression of TLR4 and TLR9 in the splenic cells from immunized mice with ZPS. And polysaccharide could also induce the expression of TLR4 of immunized splenic cells with ZPS *in vitro*.

***Keywords*:** zwitterionic polysaccharide; conjugate; immune response; splenic cells; toll like receptor

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Germinal center T cells are unique regulators that support B cell response but suppress other T cell function

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Germinal center (GC) is specialized in B-cell response and void of other immune activities. However, how B-cell response is optimized in the GC and avoids interference by other immune cells remains an enigma. Here we describe a mechanism that controls the GC environment and purges away immune deviation. We found that CD4+CD57+ T-cells, which are the major helper T-cells in GCs, actively suppress other CD4+ T-cells by cognate contact-dependent mechanism and by soluble factors including TGF-β and IL-10. CD4+CD57+ T-cells are phenotypically and mechanistically distinct from other T-cells with regulatory function. These results demonstrate that CD4+CD57+ T-cells are a novel population of regulatory T-cells that exert differential effects on B-cells and other T-cells in the same local environment.

***Keywords*:** T helper; germinal center; antibody response; immune regulation; regulatory T cells

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CABYR is a novel cancer-testis antigen in lung cancer

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CABYR is a calcium-binding tyrosine phosphorylation regulated fibrous sheath protein initially reported to be testis-specific and subsequently shown to be present in brain tumors. Our study was to determine whether CABYR is a novel cancer-testis antigen in lung cancer. RT-PCR showed that CABYR-a/b and -c presented a restricted normal tissue expression, being observed only in testis. However, mRNA expression of CABYR-a/b and -c was observed, respectively, in 13 and 15 of 36 lung cancer tissues, as well as in 3 and 5 of 14 cancer cell lines, whereas neither of them was observed in the paired adjacent noncancerous tissues or the normal cell line. High titer IgG antibodies specific to CABYR-a/b and -c were detected, respectively, in 11% (19/174) and 9% (16/174) sera from lung cancer but not in the sera from healthy donors. These results suggest that CABYR is a novel cancer-testis antigen in lung cancer and may be a promising target for immunotherapy for lung cancer patients.

***Keywords*:** CABYR; cancer-testis antigen; lung cancer; immunotherapy

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Oxidative modification mediates interaction with Apaf-1, auto-cleavage and activation of Caspase 9

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Caspase 9 is responsible for the execution of apoptosis in the extrinsic as well as intrinsic apoptotic stimuli-induced

processes. Before it exerts function, Caspase 9 itself has to undergo proteolytic activation by the mechanisms of induced–proximity and binding with Apaf-1 in the cytochrome c/dATP-dependent way. Intracellular reactive oxygen species (ROS) and redox state are increasingly reported to regulate apoptosis. There are emerging evidences that various apoptotic stimulators such as staurosporin and ceramide can induce Caspase 9 activation with accompanying ROS elevation. On the other hand, those factors triggering oxidative stress, for example, ultraviolet radiation and ionization irradiation, inevitably induce Caspase 9 activation. However the direct association of ROS and Caspase 9 activation has not been demonstrated, and little is known about the mechanisms by which ROS may facilitate and even induce Caspase 9 activation. The present study aimed to elucidate the role of ROS in Caspase 9 activation and possible mechanisms by which ROS mediates Caspase 9 activation. The results firstly showed that redox state regulated Caspase 9 activation, because Caspase 9 activation initiated by various apoptotic stimulus staurosporin or TNF-α was facilitated in an oxidative environment which resulted from Mn-SOD knockdown cells, while it was weakened, in cellular reductive environment caused by NAC. We then used hydrogen peroxide (H_2O_2) to directly mimic redox alteration, finding that H_2O_2 independently caused the auto-cleavage and activation of Caspase 9. Interestingly, the thiols of procaspase 9 were oxidized in this process. H_2O_2 also led to formation of the Caspase 9/Apaf-1 complex, while thiols-reducing agent DTT could separate the complex, suggesting that H_2O_2 resulted in oxidation of procaspase 9 and formation of intermolecular disulfide bond between procaspase 9 and Apaf-1. Because cytochrome c releasing from mitochondrion was found increased when cells exposed to H_2O_2, we reconstituted an *in vitro* mitochondrion depletion system. The result showed that thiol-specific oxidant diamide could induce Caspase 9 activation in mitochondria-free system, demonstrating that the processing and activation of oxidized procaspase 9 occurred independently of cytochrome c increase induced by H_2O_2. Then in an *in vitro* Caspase 9/Apaf-1 recombinant system, diamide caused disulfide-mediated interaction with Apaf-1, auto-cleavage and activation of Caspase 9. Taken together, procaspase 9 can be oxidized by certain amount of ROS, and the consequent oxidative modification of procaspase 9 can mediate its interaction with Apaf-1, auto-cleavage and activation. We speculate that this may constitute the basis for that ROS facilitate as well as induce apoptosis.

Keywords**:** oxidative modification; ROS; Caspase 9; Apaf-1

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Macrophage migration inhibitory factor is induced by dengue virus infection of A549 cells via nuclear transcription factor NF-κB activation

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Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine, which is significantly increased in severe dengue virus (DV)-infected patients. However, the mechanism that induces MIF production during dengue infection is still unclear. In this study, we found MIF was induced in DV-infected human immune cell lines such as Jurkat, K563, and THP1 but not in human hepatoma cell line HepG2. DV could also infect human lung epithelial cell A549 and induced MIF production in a dose and time dependent manner. RT-PCR showed MIF RNA was increased in DV-infected cells. Increase of MIF mRNA expression by DV infection was further supported in A549 cells trasfected with MIF promoter construct with luciferase reporter gene. DV infection of A549 cells also induced NFκB activation and inhibition of NFκB activation by dexamasasome or curcumin could inhibit MIF production in DV-infected cells. Taken together, these results suggest that increase of MIF mRNA and protein synthesis in DV-infected cells involve NFκB dependent signal pathway.

Keywords**:** cytokine; inflammation; signal transduction; infection; cell culture

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Skin aging and immunosenescence: biological targets

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Aging of the skin and the loss of its biomechanical properties are linked to the damages caused by an overproduction of excess free oxygen radicals (ROS) and a deregulation of the cytokines network. Disappearance of skin tone luminosity in aged skin is linked to modification in the melanogenesis pathway due to overactivity of tyrosinase and pro-inflammatory mediators. The syntheses of IL4, IL10 and IL8 in the epidermis are particularly linked to skin immunosenescence. The observed pro-inflammatory state results in the attraction of dermal macrophages and neutrophils with proteolytic activities, which could ex-

plain from the aging of the skin. We also showed recently that the concentrations of metallothioneins, zinc proteins involved in nuclear DNA protection and repair, are subnormal in the skin of asian women chronically exposed to sunlight. ELISA studies showed that an original complex of polyphenols and triterpenes extracted from orchids roots inhibited the peroxidation of cell membrane lipids and the production of the cytokine IL8 by preconfluent cultures of normal human keratinocytes stimulated with interleukin beta 1. A 0.5 % mixture inhibited production by 23 % and a 1 % mixture by 34%. Human peripheral blood cells prepared from three donors were stimulated by incubating them for 72h with a mixture of PMA (10 ng/ml) and PHA-P (10 μg/ml), with or without the molecular from orchid extract. The extract inhibited the secretion of IL4 in a dose dependent manner (0.1 % inhibited secretion by 17 % and 1% by 29 %). We used an in-house quantitative low-density microarray technique (OLISA, oligosorbent array) to show that normal human keratinocytes incubated with the extract for 24 h increased their expression of the genes encoding metallothioneins 1 & 2 and the anti-inflammatory cytokine IL10, which is also important for tissue function longevity. Tyrosinase assay based on its dopa-oxidase activity showed a dose response inhibition (up to-40%) by the mixture. All these data confirm the importance of immunosenescence in skin aging and that, by acting on specific targets linked to the immuno-inflammatory skin state, a specific orchid extract is a potential new skin anti-aging pharmacological agent.

Keywords**:** skin aging; immunosenescence; cytokines; Inflammation; orchid extract

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Critical role of IgM-mediated signals in maintaining peripheral lymphoid homeostasis

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IgM and IgD are the two antigen receptors co-expressed on the majority of mature B cells. However, it has been a long-existing question why the same B cells need to express two isotypes of immunoglubins with identical antigen specificities and whether membrane IgM and IgD play differential roles during B cell development and differentiation. Here we show that IgM-deficient mice exhibit an abnormal expansion of B-lymphocytes in the periphery. In the absence of IgM, IgD-expressing B cell subsets including T1, T2, mature follicular B cells and marginal zone B cells in the spleen of IgM-deficient mice were markedly expanded. The mechanism responsible for the accumulation of lymphocytes in the periphery appears to be associated with enhanced peripheral clonal expansion and survival rather than increased bone marrow output. Our results demonstrate that IgM is indispensable in maintaining peripheral lymphoid homeostasis and predominant expression of IgD in the absence of IgM tips the balance required for equilibrium of lymphoid system, leading to lymphoproliferation.

Keywords**:** IgM; immune regulation; B lymphocytes; homeostasis

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Combined transfections of both EBV-special epitopes and their presentation molecular HLA-A2 are more effective in promoting CTL lysis of nasopharyngeal carcinoma than those of separate transfection

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To promote special Cytotoxic T lymphocyte (CTL) lysis is a promising strategy in cancer therapy. In the present study, we examined the boosting effect of CTL upon autologous lymphoblastoid B cell lines (LCL) transfected by diverse plasimids, in order to explore the possible CTL-based immunotherapy of Nasopharyngeal Carcinoma (NPC). FCM analysis displayed rather high ratio (>30%) of successfully transfected LCLs by utilizing the DMRIE-C kit, which is especially designed for the transfection of suspended cell lines. CTL assays demonstrated that substantially higher ratio of CTL special lysis was observed upon the LCL transfected with both expression vectors encoding EBV-special epitopes and their presentation pCDNA3-HLA-A_2, in contrast to those transfected separately. By transfecting the vector encoding the presentation molecular HLA-A_2 merely, only the LCL of HLA-A_2^+ donors elicited markedly higher CTL lysis. CTL assays also showed that there existed no marked differences upon transfection by either different vectors (pCDNA3, pNGVL3 or pNGVL3-hFlex), or different EBV-derived peptides (LMP_2Pep1 or LMP_2Pep2), or with or not the doubled DNA sequence encording peptides. This study for the first time reports a promising immunotherapy strategy on NPC through boosting and eliciting the EBV-specific CTL activation by transferring vectors encoding both EBV-specific epitopes and their presentation molecular HLA-A_2 into autologous LCL, the presentation cell of MHC/peptide tetrameric complex.

Keywords**:** nasopharyngeal carcinoma (NPC); lymphob-

lastoid B cell lines (LCL); MHC/peptide tetrameric complex; cytotoxic T lymphocyte (CTL)

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Drosophila TAB2 is required for the immune activation of JNK and NF-kappaB

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The TAK1 plays a pivotal role in the innate immune response of *Drosophila* by controlling the activation of JNK and NF-κB. Activation of TAK1 in mammals is mediated by two TAK1-binding proteins, TAB1 and TAB2, but the role of the TAB proteins in the immune response of *Drosophila* has not yet been established. Here, we report the identification of a TAB2-like protein in *Drosophila* called dTAB2. dTAB2 can interact with dTAK1, and stimulate the activation of the JNK and NF-κB signaling pathway. Furthermore, we have found that silencing of dTAB2 expression by dsRNAi inhibited JNK activation by peptidoglycans (PGN), but not by NaCl or sorbitol. In addition, suppression of dTAB2 blocked PGN-induced expression of antibacterial peptide genes, a function normally mediated by the activation of NF-κB signaling pathway. No significant effect on p38 activation by dTAB2 was found. These results suggest that dTAB2 is specifically required for PGN-induced activation of JNK and NF-κB signaling pathways.

***Keywords*:** *Drosophila*; TAB2; innate immunity

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Dendritic Cells Activated with CPG-ODN 2006 Enhance the Cytotoxicity of Cytokine Induced Killer Cells Against Prostate Carcinoma

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To investigate the efficacy of Dendritic Cells (DC) activated by CpG-ODN 2006 co-cultured with Cytokine induced killer Cells (CIK) on cytotoxicity against tumor cells. Methods: Peripheral blood mononuclear cells (PBMC) isolated from healthy donors. DC can be induced from adherent cells by IL-4 and GM-CSF after 3 h incubation of PBMC and CIK can be generated from non-adherent cells. On the third day, different concentration of CpG-ODN 2006 was administered to DC cultured in vitro for five days. Mature DC can be harvested after 8-9 days incubation and then co-cultured with CIK at a ratio of 1:20. After co-culturing DC and CIK for five days, the expansion rates surface markers (CD3CD56 antigen and CD25 antigen) were evaluated by FCM. The cytotoxicity activity was investigated in cck-8 against Pc-3, DU145 prostate carcinoma cell line. Results: The colonies and expansion rates of the co-cultures CIK and DC activated with CpG-ODN 2006 are more than those of co-cultured CIK and DC without CpG-ODN 2006, and much more than those of CIK alone. The cytotoxicity of co-cultured CIK and DC activated with CpG-ODN 2006 against prostate carcinoma cell lines Pc-3 , DU145 is strongest compared to those of co-cultured CIK and DC without CpG-ODN 2006 ,and CIK alone. Conclusions: CpG-ODN 2006 was a effective adjuvant to induce Th1-dominated immune response. DC activated with CpG-ODN 2006 can strongly enhance the cytotoxicity of CIK against to prostate carcinoma cell line Pc-3 and Du145.These kind of co-cultured cells are highly effective immune cells.

***Keywords*:** dendritic cells; cytokine induced killer cells; CpG-ODN 2006; prostate carcinoma ; immune therapy

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Effect of *Pinus massoniana* Bark extract on IFN-γ-induced ICAM-1 expression in HaCaT human keratinocytes

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Intercellular adhesion molecule-1 (ICAM-1) mediates leukocyte and kerationocyte interactions. The up-regulation of ICAM-1 expression in keratinocytes correlates with various inflammatory skin diseases. Chinese *Pinus massoniana* bark extract (PMBE) with known anti-oxidant activity is comprised of various flavonoids. However, the anti-inflammatory properties and mechanism of PMBE have not been described. We found that PMBE contains 1.27 % taxifolin

and 0.55 % epigallocatechin galloate (EGCG), both well-studied plant extract with known anti-inflammatory activity. Therefore, in the present study, we evaluated the effects of PMBE, taxifolin, EGCG and mixture of taxifolin and EGCG on ICAM-1 expression. Treatment of HaCaT cells with 1000 U/ml IFN-γ for 24h markedly increased ICAM-1 expression. However, PMBE pre-treatment (40 μg/ml for 24h) significantly inhibited IFN-γ -induced ICAM-1 expression. In equal concentrations of taxifolin and EGCG, PMBE-mediated inhibition of ICAM-1 mRNA and protein expression was greater than taxifolin and EGCG-mediated inhibition. When cells were treated with three compounds at a concentration of 40 μg/ml, PMBE-mediated inhibition of ICAM-1 mRNA also was greater than taxifolin and EGCG-mediated inhibition. But the inhibition of PMBE on IFN-γ -induced ICAM-1 expression was not through NF-κB signal pathway. Previous studies indicate that PMBE including additional bioactive compounds may possibly synergize to inhibit transcription and translation of inducible ICAM-1 expression and PMBE was greater than monomeric flavonoid taxifolin, EGCG and mixture of taxifolin and EGCG. These results indicate that PMBE exhibits great potential as a therapeutic treatment for inflammatory skin diseases.

Keywords**:** *Pinus massoniana* bark extract (PMBE) ; monomeric flavonoid taxifolin and EGCG; human keratinocytes line HaCaT cells; ICAM-1; NF-κB

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NK cells produced IFNgamma and facilitated T cell activation and the generation of cytotoxic T cells in EBV infected cord blood cell cultures

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Epstein-Barr virus (EBV)-specific immunity is not transferred from mother to child. Therefore, cellular interactions in primary infection can be studied in cord blood mononuclear cell (CBMC) population. We found that the immunomodulators potentiated NK and T cell activation in the EBV infected cultures. B cell growth induced by EBV was inhibited in these cultures. NK cells are known be involved in the development of adaptive immunity. We tested therefore the EBV infected CBMC that NK cells were depleted. Methods: Thymidine incorporation assay to monitor the B cell outgrowth induced by EBV; SAP expression in immunoblot for detection of T cell activation; Flow cytometry to detect intracellular cytokines; ELISA to analyze the cytokine content in the culture media; chromium release assay and ELISPOT to detect cytotoxic function of T cells. Results: NK cells produced IFNgamma in the infected and immunomodulator containing cultures. T cell activation, B cell growth inhibition and the production of IL15 and IL12 were downregulated in the cultures devoid of NK cells. These effects could be restored by addition of IFNgamma. Cytotoxic T cell precursors were present in the EBV infected cultures, but only if the initial population contained NK cells. Conclusion: IFNgamma production of NK cells had a pivotal role in the activation of T cells and generation of cytotoxic T cells. Our results suggest the following scenario: EBV infected and activated B cells. These were recognised by NK and T cells. NK cells produced IFNgamma and T cells were primed. PSK and Trx80 activated the monocytes which then produced cytokines IL-15 and IL-12 respectively in the presence of the primed T cells and IFNgamma. The posed T cells were activated by both cytokines for function. This strategy may be exploited for generation of T lymphocytes inhibiting the proliferation of EBV infected B cells.

Keywords**:** NK cell; T cell; IFNgamma; EBV; cord blood

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Mechanism of action of anti-CD20 antibodies on non-Hodgkin's lymphoma cell lines

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Monoclonal antibodies have provided an alternative approach to treat malignant diseases. With targeting of cell surface markers, they are more specific than traditional chemotherapeutic approaches, and less systemically toxic and myelosuppressive than chemical drugs. The antitumor activities of monoclonal antibodies include anti-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), inhibition of cell proliferation, and induction of apoptosis. Rituximab is a monoclonal antibody against CD20 antigen on the surface of normal and malignant human B cells. Despite the success of chimeric CD20 monoclonal antibody (mAb), rituximab, in the treatment of non-Hodgkin lymphoma (NHL), the mechanisms are not fully understood. To better elucidate the action of anti-CD20 mAbs and develop more potent reagents, we have constructed a new mouse/human anti-CD20 monoclonal antibody, TGLA. *In vitro* studies, TGLA was similar to rituximab in terms of antigen-binding specificity and

binding avidity. TGLA also bound strongly to CD20+ cells. Although TGLA could mediate complement-dependent cell lysis of human B-lymphoid cell lines (CDC), inhibit B-lymphoid cell growth, the functions of TGLA were inferior to rituximab's. On the other hand, TGLA has shown more powerful ADCC for CD20+ cells than rituximab. *In vivo* therapy studies were performed in nude mice bearing Raji xenografts. The chimeric antibody, TGLA alone yielded median survival increases of up to 1.7-fold compared with control mice, which is similar to rituximab. Concurrent TGLA administration effectively inhibited tumor progression of lymphoma-bearing mice compared with Rituximab treatment alone. Thus these results suggest that ADCC is the central effector mechanism of anti-CD20 antibodies.

***Keywords*:** chimeric antibody; CD20; B lymphoma; cytotoxicity; rituximab

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Mgat5 specific shRNA suppress the growth of mammary adenocarcinoma cells *in vivo* and stimulating Th1 cells activation

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Golgi β1, 6N-acetylglucosaminyltransferase V (Mgat5) is required in the biosynthesis of β1, 6GlcNAc-branched N-linked glycans attached to cell surface and secreted glycoproteins. Amounts of Mgat5 glycan products are commonly increased in malignancies, and correlate with disease progression. In this study, a Mgat5 specific-shRNA eukaryotic expression vector which can efficiently downregulate the level of mouse Mgat5 was constructed and selected by RT-PCR and FITC-L-PHA labeling flow cytermetry analysis. The mgat5 specific-shRNA and control shRNA were transfeted into mammary adenocarcinoma cells MA 782 and then planted into 8-weeks BalB/C mice. We found that mgat5-specific shRNA could suppress mammary adenocarcinoma tumor cells growth *in vivo*. Mgat5-specific shRNA transfected Ma 782 cells stimulated CD4T cells proliferations. And Th1 cells and macrophages were activated in Mgat5-shRNA knockdown mice. The levels of TNF-α were significantly increased in Mgat5-shRNA knockdown mice, and the level of IFN-γ were also enhanced in CD4T cells, but the level of IL-4 was not changed significantly. RT-PCR showed that the expression of transcription factor T-bet of Th1 cytokine was increased as well. We propose that Mgat5 modified N-glycans on tumor surface may regulate Th1 cell activation.

***Keywords*:** Golgi β1, 6N-acetylglucosaminyltransferase V (Mgat5); mammary adenocarcino ma cells; Th1 cells

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Effects of ornidazole on releases of allergic mediators

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To study effects of ornidazole on releases of allergic mediators of mice by using passive degranulated test of the mast cell *in vitro*. Method Two Wistar mice were decapitated and the adiminal cavity of them was washed with 10 ml Hank'solution respectively and the solutions were centrifuged and the deposits were re-suspended in 1 ml Hank' solution. The suspensions of mast cell were mixed, divided and put into test tubules, one of them contained 50 μl suspension. Then, 10^{-5}M, 10^{-6}M, 10^{-7}M, 10^{-8}M, 10^{-9} M ornidazole were added into the test tubules and they were incubated at 37 ºC for 2 min and the reactions were terminated. Agent 48/80 was added to them and mixed. The solutions were dropped on glass slides treated with neutral red and all-degranulated, semi-degranulated and non-degranulated cells in one vision field were counted. Result Inhibitory rates of 10^{-5}M, 10^{-6}M, 10^{-7}M, 10^{-8}M, 10^{-9} M ornidazole were respectively 64.9 %, 59.8 %, 48.6 %, 41.8 %, 31.7 %. Conclusion Ornidazole is a anti-protozoon and anti-anaerobe agent with ornidazole and it is mainly used for the infections by anaerobia, amiba, giardia and trich ornidazole omonad. Ornidazole is a special among the anti-infection agents. The results of this study demonstrated that ornidazole may inhibit degranulation of the mast cell significantly and its inhibition is dose-dependent. According to the results, we can know thet ornidazole may produce anti-inflammatory and anti-immune effects besides its antimicrobial activities.

***Keywords*:** allergic; mediators; ornidazole; rffects; releases

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Steroid receptor coactivator 3 is a translational repressor and a negative regulator of inflammation

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Steroid receptor coactivator 3 (SRC-3) is a member of the SRC/p160 family of transcription coactivators for nuclear receptors and other transcription factors. Although multiple physiological roles of SRC-3 have been revealed, its involvement in the inflammatory process remains unknown. Here we show that mice lacking SRC-3 are hypersensitive to LPS-induced lethality. In these animals, LPS induces higher levels of proinflammatory cytokines such as TNF-alpha, IL-6 and IL-1 compared to wild-type animals, which contributes to the increased lethality. *In vitro*, peritoneal macrophages lacking SRC-3 produce significantly more TNF-alpha, IL-6 and IL-1 proteins than wild-type controls, although they express similar amounts of cytokine mRNAs, suggesting that SRC-3 can exert effects at post-transcriptional levels. The lack of SRC-3 significantly increases the proportion of TNF-alpha mRNA that associate with polysomes, suggesting that SRC-3 can function as a translational suppressor. Overexpression of SRC-3 inhibits the expression of a reporter construct containing TNF-alpha 3' untranslated region (UTR) with intact adenylate uridylate-rich elements (AREs), but has no effect on the same construct with AREs deleted, thus indicating that the translational repressive effect of SRC-3 is dependent on AREs. The association of SRC-3 with translation repressors TIA-1/TIAR suggests that SRC-3 cooperates with other translation repressors to regulate the cytokine mRNA translational initiation. In summary, our data demonstrate that SRC-3 is a novel player in cytokine regulation and a potential new target for anti-inflammatory therapy.

***Keywords*:** steroid receptor coactivator; translational repressor; inflammation

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Human HLA-G1 inhibits natural killer cytotoxicity through blocking the activating signal transduction pathway and formation of activating immunological synapse

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Non-classical MHC class I molecule Human Leukocyte Antigen (HLA)-G is normally expressed on the placental cells, especially fetal endothelial cells and invasive cytotrophoblast cells at the maternal-fetal interface, and meditates immune tolerance in pregnancy through interaction with immune cells including natural killer (NK) cells. In this study, we investigated the mechanisms underlying HLA-G1-mediated inhibition of NK cytotoxicity using HLA-G1-transfected K562 cells and NK92 cells. We found that inhibition of NK cytotoxicity by HLA-G1 was associated with a decreased formation of NK-target cell conjugates and defective formation of immunological synapse, as characterized by actin depolarization and perforin immobilization in activating NK cells. HLA-G1 engagement induced dephosphorylation of Vav by tyrosine phosphatase-1 (SHP-1), and thus blocked Syk to MEK/ERK activating signaling pathway in activating NK cells. These results indicate that HLA-G1 inhibits NK cytotoxicity through blocking activating signal transduction pathway, which is required for the formation of activating immunological synapse.

***Keywords*:** HLA-G1; natural killer cells; cytotoxicity; signal transduction; immunosynapse

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Hydrogen sulfide inhibits nitric oxide production and nuclear factor-κB activation via heme oxygenase-1 expression in RAW264.7 macrophages stimulated with lipopolysaccharide

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Hydrogen sulfide (H_2S), a regulatory gaseous molecule that is endogenously synthesized by cystathionine γ-lyase (CSE) and/or cystathionine β-synthase (CBS) from L-cysteine (L-Cys) metabolism, is a putative vasodilator, and its role in nitric oxide (NO) production is unexplored. Here, we show that at noncytotoxic concentrations, H_2S was able to inhibit NO production and inducible NO synthase (iNOS) expression via heme oxygenase (HO-1) expression in RAW264.7 macrophages stimulated with lipopolysaccharide (LPS). Both H_2S solution prepared by bubbling pure H_2S gas and NaSH, a H_2S donor, dose dependently induced HO-1 expression through the activation of the extracellular signal-regulated kinase (ERK). Pretreatment with H_2S or NaHS significantly inhibited LPS-induced iNOS expression and NO production. Moreover, NO production in LPS-stimulated macrophages that are expressing CSE mRNA was significantly reduced by the addition of L-Cys, a substrate for H_2S, but enhanced by the selective CSE inhibitor β-cyano-L-alanine but not by the CBS inhibitor aminooxyacetic acid. While either blockage of

HO activity by the HO inhibitor, tin protoporphyrin IX, or down-regulation of HO-1 expression by HO-1 small interfering RNA (siRNA) reversed the inhibitory effects of H_2S on iNOS expression and NO production, HO-1 overexpression produced the same inhibitory effects of H_2S. In addition, LPS-induced nuclear factor (NF)-κB activation was diminished in RAW264.7 macrophages preincubated with H_2S. Interestingly, the inhibitory effect of H_2S on NF-κB activation was reversed by the transient transfection with HO-1 siRNA, but was mimicked by either HO-1 gene transfection or treatment with carbon monoxide (CO), an end product of HO-1. CO treatment also inhibited LPS-induced NO production and iNOS expression via its inactivation of NF-κB. Collectively, our results suggest that H_2S can inhibit NO production and NF-κB activation in LPS-stimulated macrophages through a mechanism that involves the action of HO-1/CO.

Keywords**:** hydrogen sulfide; heme oxygenase-1; nitric oxide; macrophage; nuclear factor-κB

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Studies on the application of cell fusion technology to the breeding of Bac.subtilis with high cellulase activity

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Cell fusion is called protoplast fusion, cell hybridization or somatic hybridization, which is a fast-breaking cell engineering technology in the past 20 years. After mediated and cultivated, cell of different species is integrated into a hybridized cell of one or more nucleus through asexual propagation by artificial method in vitro. In this paper, cell fusion method was adopted in the interspecific hybridization of parents-*Bac.subtilis*(abbreviated as BS) and *Tri. viride* (abbreviated as TV) by associated inducement which was He–Ne laser and polyethyleneglycol (abbreviated as PEG), and inactivated protoplast technique in order to cultivate hybridized strains of BS with high cellulase activity. After mixed and affected by PEG, protoplasts got sticky and touched each other furthest because of the descent of cell's surface tension. Tiny holes were formed between cells by laser and cell content was exchanged through these tiny holes. Because these temporary holes formed by laser could be repaird, cells could be separated from each other and tiny holes disappeard after washing, which could prevent cell content from overflow and accomplish the process of hybridization. The results showed that: the best zymolysis conditions for BS and TV protoplasts release and regeneration through orthogonal experiment were as follows: 1mg·mL^{-1} lysozyme treated BS protoplasts for 1.5 h at 37 ºC and 4 mg·mL^{-1} lywallzyme treated TV protoplasts for 2 h at 30 ºC. In these conditions, the number of protoplasts release was respectively 8.87×10^8 units/mL and 8.62×10^7 units/mL and the regeneration rate was respectively 54.1% and 29.3%. After mixing two kinds of protoplasts and adding 1 mL 40% PEG(6000) pre-heated at 50 ºC into the mixture, they were irradiated by He–Ne laser for 4 min, which was the associated inducement by laser and PEG, the fusion rate got the highest which was 1.23×10^{-5}. The fusants with fine characteristics was selected and cultivated through initial and repeated sieving. Fusants were tested by the esterase isoenzyme patterns, enzyme-producing abilities and hereditary stability. The results showed that the fusants inherited the fine features from their parental strains, which indicated that they were new strains produced by the fusion of the parental strains. Among these fusants, the 16# hybridized strain had highest cellulase activity —79.8 U/mL.

Keywords**:** cell fusion; *Bac.subtilis*; cellulase; interspecific fusion; isoenzyme

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Effect of ornidazole on releas of allergic mediators

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To study effects of Ornidazole on releases of allergic mediators of mice by using passive degranulated test of the mast cells *in vitro*. Method: Two Wistar mice were decapitated and the abdominal cavity of them was washed with 10 ml Hank' solution respectively. After centrifugalization, the deposits were re-suspended in 1 ml Hank' solution. The suspensions of mast cells were mixed, divided and put into test tubes which contained 50 μl suspension respectively. Then, 10^{-5}M, 10^{-6}M, 10^{-7}M, 10^{-8}M, 10^{-9}M ornidazole were added into the test tubes and they were incubated at 37 ºC for 2 min and the reactions were terminated. Agent 48/80 was added to them and mixed. The solutions were dropped on glass slides treated with neutral red and all-degranulated, semi-degranulated and non-degranulated cells in one field of vision were counted. Result Inhibitory rates of 10^{-5}M, 10^{-6}M, 10^{-7}M, 10^{-8}M, 10^{-9}M ornidazole were 64.9 %, 59.8 %, 48.6 %, 41.8 %, 31.7 % respectively. Conclusion: Ornidazole is a kind of nitroimidazole drugs with anti-protozoon and anti-anaerobe fuction. It is mainly used for the infections by anaerobia, amebic protozoa, giardia and trichomonad. Ornidazole has its unique feature among

anti-infectives. The results of this study demonstrated that ornidazole inhibit degranulation of the mast cell significantly and its inhibition is dose-dependent. According to the results, we can know that ornidazole may produce anti-inflammatory and anti-anaphylaxis effects besides its antimicrobial activities.

***Keywords*:** allergic reaction; sensitization

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Preliminary analysis of voltage-dependent potassium channels in human peripheral blood monocytes

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To analyse the property of voltage-dependent potassium (*Kv*) channels in healthy people's peripheral blood monocytes. Methods: Peripheral blood mononuclear cells were isolated by Ficoll density gradient centrifugation; monocytes were separated from lymphocytes by the adherence technique. The whole-cell patch-clamp technique was used to record K^+ currents, through EPC-10（HEKA, Germany）amplifier. A typical pulse protocol clamped the cell at a holding potential of –40 mV and stepped in 20 mV increments from –80 to +60 mV, with each pulse lasting 450 ms at an interval of 1 s Results: The mean membrane capacitance (Cm) of monocytes is (2.66±0.83) pF. We abserved whole cell currents, which were time-independent and out-ward. To analyze the currents and the current-voltage relation, we infered that the currents were delayed rectifying K^+ currents (Kdr). Conclusion: Kdr are expressed in human normal peripheral blood monocytes.

***Keywords*:** monocytes; potassium channels; patch-clamp technique

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Modified heparin RO-heparin can inhibit β2-integrin-mediated Acute Inflammation

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The adhesion of leukocytes to vascular endothelium is important to the generation of inflammatory responses. Three major adhesion molecules families, namely, selectins, β2-integrins, immunoglobulin-like cell adhesion molecules play a major role in the process. Several studies have shown that heparin and some modified heparin can inhibit selectins-mediated acute inflammation. These heparin derivatives while has lost or partly lost its anticoagulant activity preserving anti-inflammatory property. Recentness, we have reported that RO-heparin can inhibit P-selectin-mediated Acute Inflammation. However selectins and β2-integrins together participate in inflammation response, RO-heparin whether also can inhibit β2-integrins-mediated cells adhesion when block the P-selectin-mediated cells adhesion in vivo, which is not clear. To address the problem, in the present study, we tested as inhibitors of the human neutrophils binding to ICAM-1, adhering to COS-7 cells expressing a transfected human ICAM-1 cDNA and human umbilical vein endothelial cells (HUVECs) under static conditions, as will as neutrophils adhering to HUVECs monolayer expressing ICAM-1 under flow conditions. The results indicated that RO-heparin also has a potent anti-β2-integrin (CD11b/CD18) activity while has lost or partly lost its anticoagulant activity. The findings suggest RO-heaprin may act as a new safer medicine for treatment of inflammation, a clinical trial to test this hypothesis is indicated.

***Keywords*:** inflammation; RO-heparin; β2-integrin; anticoagulant activity; human umbilical vein endothelial cells (HUVECs)

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The polymorphism of HLA-A/B/DR/DQ gene of Han nationality in Zunyi and its association with EHF

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Objective: The epidemic hemorrhagic fever (EHF) is a kind of acute natural epidemic focus disease caused by Hantavirus. China is one of the countries seriously harmed by EHF, which has been one of the most harmful virulent diseases besides the virus hepatitis, so it has been classified as the momentous project of diseases prevention and control in China. Zunyi is one of the regions with high-frequency

of this disease. We have explored the association between the HLA-A/B/DR/DQ allelic polymorphism and EHF in Han nationality in Zunyi area, and have studied the immunopathogenesis of EHF on immunogenetics view, trying to offer the new clue for immunoprophylaxis, immunotherapy and immunoregulation of EHF and simultaneously understand the distribution of allele polymorphism of the HLA-A/B/DR/DQ of Zunyi Han Chinese at gene level to obtain corresponding data of genetics and anthropology. Methods: The HLA-A/B/DR/DQ locus was genotyped by polymerase chain reaction-sequence specific primers (PCR-SSP) technique for 100 patients with EHF and 100 healthy controls, unrelated Han population whose families have lived in Zunyi for more than 3 generations and every allelic frequency was comparatively analysed with that of some main Chinese and some overseas populations. Results: The allele frequencies of HLA-A*31, -B*58, and -DRB1 *16 in patients with EHF were markedly higher than those in control group (P<0.05), while the allele frequency of HLA-B*40 notably decreased (P<0.05).And there was not significantly different between the patients with the control in the AF of HLA-DQB1 alleles. We have respectively obtained 12 HLA-A alleles, 25 B* alleles, 13 DRB1* alleles and 7 DQB1* alleles at low-middle resolution level. Conclusions: The results indicate that HLA-A*31, -B*58, and -DRB1*16 alleles are the positive association to the patients of EHF among Zunyi Han Chinese, which might have susceptible function or be linked to the really susceptible gene. While HLA-B*40 is the negative association to the patients of EHF among Zunyi Han Chinese, and it is also suggested that the expression of HLA-B*40 might be associated with an antagonist effect in the pathogenesis of EHF. Immunogenetics factors may be participate in the pathogenesis of EHF; Zunyi Han Chinese may be closely national integration into Chongqing Han Chinese, and it belongs to Southern Han Chinese, simultaneously Zunyi Han Chinese have their own territory feature with such as high-frequency allele HLA-B*07 and low-frequency allele HLA-DRB1*10.

***Keywords*:** epidemic hemorrhagic fever (EHF); HLA-A/B/DR/DQ genotyping; allele frequency (AF); polymerase chain reaction-sequence specific primers (PCR-SSP)

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Functions and mehcanism of IL-17 in cerebral ischemia

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Cerebral infarction is one of the dominant reasons of cerebral ischemic injury. Increasing evidences showed that the inflammatory response exacerbates the cerebral ischemic injury. Inflammatory cells and proinflammatory cytokines play an important role in the inflammatory exacerbation. Recently studies showed that IL-17 and IFN-γ mRNA levels were elevated in the ischemic hemispheres of pMCAO-operated rats. These studies suggested that altered levels of IL-17 may affect outcome of brain ischemia. The function and mechanism of IL-17 in ischemic cerebral injury is still unkown. In this study, pathological changes, the expression of IL-6, IL-1, IL-10 and IL-17 in the pMCAO-operated SD rats administered IL-17 via tail vein were investigated. Levels of IL-6 in ischemic hemispheres of MCAO and MCAO/IL-17 groups were elevated significantly and peaked at 24 h and 48h. The IL-6 levels in ischemic hemisphere of MCAO/IL-17 group were significantly higher than that of MCAO group (P<0.001). Levels of IL-10 in ischemic hemispheres of MCAO and MCAO/IL-17 groups were elevated significantly and peaked at 24 h and 48 h. The IL-10 levels in ischemic hemisphere of MCAO/IL-17 group were significantly lower than that of MCAO group at 24 h, 48 h, and 6 d (P<0.05). Our results suggested that the expression of IL-6 had been up-regulated, whereas the expression of IL-1 and IL-10 had been down-regulated by external administered IL-17. Levels of IL-17 in ischemic hemispheres of MCAO and MCAO/IL-17 groups were elevated delayingly and peaked at 6d and 8d. The IL-6 levels in ischemic hemisphere of MCAO/IL-17 group were slightly lower than that of sham group at 24 h, 48 h, and 6 d (P>0.05), and significantly lower at 8 d (P <0.001). There was no significant difference between the IL-17 levels of MCAO/IL-17 and MCAO groups, suggested that the internal IL-17 expression had been slightly down-regulated.To demonstrate the cell types which expressed IL-17, the ischemic cerebral sections of pMCAO-operated rats had been detected by GFAP and IL-17 double-staining. Results showed only GFAP-stained cells could be seen in the normal hemisphere of MCAO rats, while a lot of GFAP and IL-17 double-stained cells could be observed in the ischemic hemisphere. This result showed that IL-17 can be produced by astrocytes if a ischemic signal stimulation exists. In conclusion, IL-17 probably protect the neural cells from inflammatory injury after cerebral ischemia by up-regulating IL-6 and down-regulating IL-1.

***Keywords*:** cerebral ischemia; IL-17; rat

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Detection method of complement analogues of coelomic fluid in *Apostichopus japonicus* by means of chemiluminescent Immunoassay (CLIA)

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A highly sensitive and specific chemiluminescent immunoassay (CLIA) method was developed for quantitative detection of complement analogues, AjC3, AjC4 in *Apostichopus japonicus* for the first time. Sheep anti-human C3, C4 antibodies were absorbed polystrene tubes wich were treated with ultraviolet. The antibodies were labeled with horseradish peroxidase (HRP). Luminol solution and H_2O_2 were used as the substrate of HRP. The most optimum concentration of antibody coated in the polystyrene tubes is 1 μg/ml. The optimum dilution level of HRP-anti-IgC3 and HRP-anti-IgC4 is 1: 2000. The stabilities of HRP-anti-IgC3 and HRP-anti-IgC4 were monitored at 4 °C over 8 days and at room temperature over 5 days. The immunoreaction reaches equilibrium after 2 h of incubation at 20 °C. The detection range of the method was 0.1—10 ng/ml and it displayed good linearity. The sensitivity of the method can be used to detect AjC3, AjC4 concentrations as low as 0.1 ng/ml. The results showed that the complement analogues, AjC3, AjC4 in Apostichopus japonicus can be detected by the method ,and the content of AjC3 is 6.58±1.4 μg/ml, and AjC4 is 0.67±0.3 μg/ml.

Keywords: *Apostichopus japonicus*; coelomic fluid; complement analogues AjC3, AjC4; chemiluminescent immunoassay

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The Arg753Gln and Arg677Trp polymorphisms of the human Toll-like receptor 2 gene and the association with tuberculosis disease in Zhejiang Han Population

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Toll-like receptor2 (TLR2) plays a critical role in immune response to mycobacteria. In order to know the TLR2 Arg753Gln and Arg677Trp single nucleotide polymorphisms (SNP) and the relationship with the developing of tuberculosis in Zhejiang Han population, we collected the blood samples from 170 TB patients compared with 199 ethnically and age-matched healthy blood donors. Polymerase chain reaction with sequence specific primer method (PCR-SSP) was applied to detect the two polymorphisms. The G/G genotype of Arg753Gln polymorphism was observed in 58.23% and 84.2% in TB patients and the controls, respectively. And the G/A genotype in the cases was much greater, 41.77%, compared with 15.8% in the controls. Statistically significant difference was found between the two groups, $P<0.001$. Moreover, no one in either group was homozygous for the mutation (A/A). Meanwhile, the Arg677Trp did not exist in the two groups. The present data suggests that the Arg753Gln polymorphism of the TLR2 gene in Zhejiang Han population has its specific distribution trait. And it influences the risk of developing tuberculosis. The polymorphism of Arg677Trp couldn't find the among the Zhejiang Han population, however, it is likely that Arg677Trp may also exist in the Han population but with a very low frequency.

***Keywords*:** Toll-like receptor2 (TLR2); Single nucleotide polymorphism (SNP); Tuberculosis

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