SESSION 3 : AUTOIMMUNITY

Molecular characterization of anti-idiotypic antibodies derived by phage display from a patient with autoimmune thrombocytopenia

P. Fischer, M .M. Uttenreuther-Fischer, N. Jendreyko and G. Gaedicke
Lab. Molecular Biology, University Children's Hospital, Charité, Humboldt-University,Berlin, GERMANY

Autoimmune thrombocytopenia (ITP) is characterized by enhanced platelet destruction; the etiology is still unknown. No specific treatment is available. Intravenous infusions of immunoglobulins (IVIG) can result in a fast recovery of normal platelet counts in the patients. Anti-idiotypic blockade of autoantibodies or interaction with the idiotypic network are possible modes of action. To analyse those patient-derived antibodies reacting with IVIG we prepared an antibody phage display library from an ITP patient in the pComb3 vector using established protocols (*Nature* **355:** 258). The library of 1.1 x 10^6 members was then biopanned on IVIG-coated ELISA plates blocked with BSA. After 4 rounds of panning we tested antibody displaying phage (phabs) supernatants of single clones (*Biotechniques* **16:** 828) directly in ELISA for IVIG-reactivity. Detection of bound phabs was done with HRP-labelled anti-M13 serum. Positive clones were reevaluated for cross-reactivity with human Fc-fragments or BSA. 14 phabs with a strong signal on IVIG compared to a very weak signal on Fc-fragments or BSA, e.g. OD 1.2, OD 0.3, and OD 0.1, respectively, were selected for preparation of soluble Fab-fragments by removing the gene-III fragment. Restriction analysis revealed that all but two of those clones had both the heavy and light chain inserts. However, three phabs that gave positive signals on IVIG, as well as Fc and BSA revealed neither antibody insert. For analysing soluble Fab-fragments derived from the same library, we coated the plates with the Fab preparations and added IVIG or Fc-fragments in the second step. Bound IVIG or Fc could then be detected with anti-human Fc-specific labelled antibodies. Although the sensitivity was low due to bacterial proteins in the Fab preparation used for coating, a specific reaction with IVIG but not with Fc-fragments was determined. Positive phab-clones are currently being DNA-sequenced. It will be interesting to determine whether those anti-idiotypic antibodies also recognize platelet antigens. Our data provide a new approach in analysing patient-derived MAbs of the IgG class reacting with therapeutically given IgG. [We thank H.Lerch for technical asssistance and Dr C. Barbas of Scripps Res. Inst. for the pComb3 vector. This work is supported by DFG grants #GA 167/5-1 and 167/6-1.]

Construction of a single-chain antibody fragment library specific for human thyroglobulin

Martine Biard-Piechaczyk, Isabelle Navarro-Teulon, Sylvie Peraldi-Roux, Majida Bouanani, and Bernard Pau
CNRS, UMR 9921, UFR Pharmacie, Montpellier, FRANCE

Thyroglobulin is a major autoantigen in autoimmune thyroid diseases such as Hashimoto's thyroiditis or Graves' disease. For a better knowledge of the autoantibody repertoire associated with these pathologies, our aim is to engineer anti-human thyroglobulin (hTg) autoantibodies. We previously showed that these autoantibodies present in the sera of patients have a restricted recognition to a particular region of the molecule of hTg, named region II. peripheral blood B lymphocytes of a patient suffering from Hashimoto's thyroiditis were purified using anti-CD19 magnetic beads and total RNA was extracted. Each step of the construction of the library has been optimized. Gene amplification is the first crucial step to obtain a highly diverse library with a good efficiency. Ten DNA polymerases were tested in the three successive PCRs using a large set of primers. Only one allows us to amplify the majority of the V gene families. After cloning in the pHEN phagemid, the vectors containing the insert were purified after specific cleavage in the scFv gene, religated and transfected in *E. coli*. We used phage display technology to enrich the library with specific anti-hTG scFv fragments. Specificity and affinity of the selected clones strongly depend on protocol of panning. Two rounds of selection were performed using either hTg adsorbed on tube or hTg coupled on glutaraldehyde activated beads. To detect anti-hTg antibody fragments, ELISA screenings were performed with scFv displayed on phage or with soluble scFv fused or not to the g3 protein. Soluble scFv fragments were obtained from bacterial supernatant or periplasmic extract. The best way appeared to be the detection of scFv fragments from periplasmic extract in 96-well plates. We obtained anti-hTg scFv fragments which are in the process of characterization. These results should participate in a better understanding of the emergence of the autoantibody repertoire.

Incidence of 'anti-mouse' antibodies in thrombocytopenic patients with autoimmune disorders

Gisèle Clofent-Sanchez, Sophie Lucas, Jeanny Laroche-Traineau, Patrick Rispal, Jean-Luc Pellegrin, Paquita Nurden and Alan Nurden
CNRS, UMR 5533, Hôpital Cardiologique du Haut Lévêque, Pessac, FRANCE

Two monoclonal antibody immobilization of platelet antigen (MAIPA) assays were peformed on sera from a series of 25 patients with thrombocytopenias associated with autoimmune disease. In the classic MAIPA assay, control platelets were incubated simultaneously with selected murine MAbs and human sera. In MAIPA II, the control platelets were incubated first with the human serum and then, after washing, with the selected mouse MAb. The comparison between these two tests allowed us to distinguish (a) autoantibodies to platelet glycoproteins (GP) IIb-IIIa, Ib-IX, Ia-IIa, IV and p24, and (b) serum antibodies recognizing the mouse MAbs to platelet glycoproteins used in the assay. We then compared the frequency of such antibodies in patients (i) with autoimmune thrombocytopenic purpura (ATP), (ii) with thrombocytopenia associated with other autoimmune disease states, and (iii) in healthy donors with a normal platelet count. Our results suggest that the nature of antibodies in thrombocytopenic patients is far more complex than we originally thought. Indeed, it is not unusual for any one patient to have formed antibodies to more than one target site and/or to possess antibodies reacting with 'anti-mouse' epitopes. Autoantibodies (both MAIPA I and II positive) or 'anti-mouse' Abs (MAIPA I positive and MAIPA II negative) were detected both in patients with classic ATP and in those where an ATP-like condition was associated with other autoimmune states. Differences in the incidence of anti-mouse antibodies in the sera of thrombocytopenic patients (56.5%) and healthy donors (10%) were statistically significant. This suggests that their production may be a relatively common event in thrombocytopenias associated with autoimmune disease. We speculate that the presence of 'anti-mouse' antibodies may reflect an abnormality in the immunological modulation of the idiotypic/anti-idiotypic network. 'Anti-mouse' antibodies here detected may represent anti-idiotypic antibodies produced against human autoantibodies and cross-reacting with their murine counterparts.

Autoimmune phenomena in patients with tropical spastic paraparesis

M.F.Smikle, E.N.Barton, O.St.C.Morgan, J.Luseko and W.A.Blattner
University of the West Indies, Kingston, JAMAICA, and NIH, Bethesda, MD, USA

The finding of antibodies to the retrovirus HTLV-1 in sera from some of the patients with certain rheumatic diseases has provoked much discussion regarding the possible etiologic role of this virus in the development of autoimmune disease. The pathogenetic role of autoreactivity in diseases caused by HTLV-1 has been investigated to a lesser extent. Hence the pathogenesis of tropical spastic paraparesis (TSP), also called HTLV-1 associated myelopathy (HAM), is still not known. In this report, serum samples from 76 consecutive Jamaican TSP patients were examined for autoantibodies and phenomena associated with autoimmune processes including hypergammaglobulinaemia (HGG), circulating immune complexes and hypocomplementaemia. The TSP patients also were assessed for clinical evidence of autoimmune disease. Compared to the general Jamaican population, statistically significantly increased frequencies of several autoantibodies were found in the TSP patients. Significant titers of organ specific and non-organ specific autoantibodies were found in the sera of 38.2% (27/76) of the patients, anticardiolipin antibodies in 29.0% (22/76) and biological false positive tests for syphilis 9.2% (7/76). HGG occurred in 90.8% (69/76) including those which were HTLV-1 seronegative, elevated levels of complement-fixing immune complexes were found in significantly higher frequency in TSP sera (57.1%) compared to sera from healthy controls (5.3%; $p < 0.01$). Except for one patient, clinical evidence of autoimmune disease was not observed in the patients with autoantibodies. This absence of clinical evidence of the disorders associated with these autoantibodies imply that they are likely to be epiphenomenal. However, they provide proof of a propensity to autoreactivity in TSP patients. The high frequency of HGG and the variety of autoantibodies are evidence of polyclonal activation of B cells. This study has not explored the full repertoire of autoantibodies which could result from the polyclonal activation of B cells. Further studies should be undertaken to ascertain the presence or absence of tissue-specific autoantibodies directed at the CNS of TSP patients, the antigenic component of the complement-fixing immune complexes and how HGG might be related to the degeneration of nerve tissue which characterizes TSP.

Characterization of the antigen binding domain of a natural human anti-F(ab')$_2$ autoantibody

M. Welschof °*, M. Little*, S. Kipriyanov*, D.Stanescu°, M. Zewe*, C. Hain°, S. Dübel*, F. Breitling*, G. Opelz° and P. Terness°
°*University of Heidelberg, Institute of Immunology and *German Cancer Research Center (DKFZ), Heidelberg, GERMANY*

The human immune system produces an IgG-anti-Ig antibody that binds to F(ab')$_2$ γ-fragments. Clinical studies indicated that IgG-anti-F(ab')$_2$ plays a role in the pathogenesis of autoimmune hemolytic anemia, kidney graft rejection, AIDs and lupus erythematosus. The aim of this work was the isolation and characterization of anti-(Fab')$_2$ autoantibodies from a phage display library generated from human IgG cDNA of a donor with a high anti-(Fab')$_2$ serum titer. The antibody library was generated from the cDNA of isolated peripheral mononuclear cells. To obtain a large repertoire or rearranged V genes, we designed a set of 15 Ig primers for PCR amplification. Sequence analyses of the PCR products confirmed that a large range of rearranged V genes was amplified. These PCR products were cloned into the phagemid display vector pSEX. First, κ and *lambda* sublibraries were created. For each sublibrary a complexity of 3 x 10^6 independent clones was achieved. In a next step the variable regions of the γ-chains were cloned into these sublibraries. The resulting library with a repertoire size of 6 x 10^6 was screened with F(ab')$_2$ γ-fragments. After five rounds of selection, we analysed 95 single clones in a phage ELISA assay: all bound to F(ab')$_2$ γ-fragments. Sequence analysis of 12 clones with high anti-F(ab')$_2$ activity revealed that 11 were identical whereas one differed by a silent point mutation in the heavy chain and three amino acid exchanges in the light chain variable region. The heavy chains belonged to the VH_3 and the light chains to the V(κ)$_2$ gene family. The 11 identical light chains were completely homologous to germline sequence DPK15. After expression and purification of soluble scFv, ELISA assays showed that the monomeric scFv fraction binds to F(ab')$_2$ but not to Fab, Fc or intact IgG. This was confirmed by surface plasmon resonance measurements that revealed high affinity (K_a= 2.8 x 10^7 M^{-1}) compared to previously described intact anti-IgG autoantibodies of rheumatoid patients. The present study defines for the first time the gene segment structure of the antigen binding domain of natural human IgG-anti-F(ab')$_2$ autoantibody and describes the binding kinetics of the purified monomeric fragments.

A sulfatide-reactive MAb, derived from a patient with MS, selectively binds to myelin structures in ultrathin sections of rat brain, as revealed by cryo-immunoelectron microscopy

E.Kirschning, G. Rutter, C. Huckhagel, I. Ellhof and H. Hohenberg
Dept for Electron Microscopy, University of Hamburg, Hamburg, GERMANY

Experimental data indicate that autoantibodies against the myelin glycolipids galactocerebroside (GalC) in sulfatide might be involved in demyelinating processes in multiple sclerosis (MS). Recently, we could show that a MAb obtained from EBV transformation of peripheral blood cells from a patient with MS, specifically binds to the surface of $GalC^+$ oligodendrocytes in mixed brain cell cultures of newborn rats (Kirschning E. *et al. J. Neuroimmunol* 1995; **56:** 191). Using a panel of selected pure lipids we could further demonstrate that this IgM-MAb, designated DS1F8, selectively reacted with sulfatides, both in a liposome agglutination assay and in an ELISA.
In the present study, DS1F8 was tested for its ability to react with myelin *in situ*, which of course would be a prerequisite for a possible pathophysiological effect *in vivo*. However, lipid antigens were extracted when the brain tissue was processed by conventional fixation and embedding procedures. For these reasons, the brain tissue was cryofixed by high pressure freezing and dehydration was carried out by cryosubstitution. Ultrathin sections of the epon-embedded native material were immunostained with DS1F8 or a murine MAb 'anti-GalC' and antibody binding sites were localized using an appropriate second antibody coupled to 10 nm gold particles. This procedure retained a good ultrastructure without any visible immobilization of myelin glycolipid antigens. Both MAbs strongly reacted with myelinated axons, while non-myelinated structures were devoid of any immunogold labelling. These data indicate that autoantibodies with binding properties similar to DS1F8 might react with myelin *in vivo* and so might be involved in demyelinating processes in the CNS.

[Supported by Gemeinnützige Hertie-Stiftung, Frankfurt/ Main, Germany.]

'Autoantibody dominance' pattern following idiotypic manipulation of naive mice by immunization with different epitope specific anti-U1RNP antibodies

Boris Gilburd, Jacob George, Arnon Afec*, Maya Damianovitch, Andrea Doria¶, Silvano Todesco¶ and Yehuda Shoenfeld
*Research Unit of Autoimmune Disease, Sheba Medical Center, and *Institute of Pathology, Tel Aviv University, ISRAEL; and ¶Cathedra E. Divisione Rheumatologia, Univ. Padua, ITALY*

Antibodies directed against the ribonucleoprotein complex (anti-U1RNP antibodies) are considered characteristic of mixed connective tissue disease (MCTD). Anti-U1RNP recognizes three main epitopes: 70 Kd, A (34 Kd) and C (22 Kd) proteins. Similar to other autoimmune diseases, it is currently unknown whether anti-U1RNP autoantibodies are by themselves pathogenic. The original aim of the present study was to induce in mice both a clinical syndrome resembling MCTD in humans and substantiate its pathogenic role by demonstrating the appearance in the mice sera, or murine anti-U1RNP autoantibodies. This hypothesis was formulated on the basis of previous studies conducted in other laboratories and ours, in which active immunization of BALB/c mice with different autoantibodies resulted in production of respective murine autoantibodies, and corresponding clinical manifestations. Three groups of BALB/c mice were immunized intradermally in the hind footpads with anti-U1RNP-IgG preparations obtained from three different patients withMCTD. Group 1 was immunized with human IgG#5 (U1-70Kd-A - positive), group 2 with IgG#9 (U1-70Kd - negative), U1-A, U1-C, B-B' - positive) and group 3 with IgG#4 (U1-70Kd, U1-A, U1-C - positive). Immunoblot assay showed that mice immunized with different human anti-U1RNP antibodies developed predominantly autoantibodies directed against U1 68-70 Kepitope. This pattern of antibody production has been designated by us as 'autoantibody dominance' and was not associated with respective clinical findings. This study suggests that idiotypic manipulation by active immunization of mice with different epitope specific human anti-U1RNP antibodies result in restricted production of murine epitope specific 68 -70 Kd autoantibodies.

Recombinant anti-rhesus antibody

L. Edelman, C. Margaritte, E. Monchâtre,
D. Blanchard°, F.-X. Deramoudt*, F. Morin,
G. Dumas and C. M. Kaczorek*

Institut Pasteur, Lab. Technol. Cellulaire, Paris;
**Proteine Performance, Montpellier; and ° CRTS*
Nantes, FRANCE

Anti-rhesus (Rh) immunoglubulins indispensable for the prevention of hemolytic disease of newborn are obtained after multiple immunization of Rh^- donors and are injected i.v. (100 µg/dose) into all Rh^- women post-partum or post abortion. Recombinant anti-Rh antibodies would provide an almost never-ending source of anti-Rh antibodies , as well as diminishing the risks of contamination by virus and other pathogens which may be present in the Ig preparations made from pooled human sera. The immunization of volunteers would also be avoided. The recombinant antibody that we made was obtained from the human monoclonal line D7C2 which secretes an IgM anti-Rh antibody. The D7C2 clone was isolated after EBV immortalization of peripheral blood lymphocytes, originating in an Rh^- donor immunized several times. The culture supernatant of this immortalized cell line agglutinated all Rh^+ red blood cell (RBC) and most of the partial and weak Rh phenotype. Nucleotide sequences coding for the variable parts of heavy and light chains of this antibody showed that the VH fragment belonged to the VH4 family and the VL fragment, to the V1(*lambda*) V family. The recombinant anti-Rh antibody was obtained by a baculovirus vector and expression in insect cells. The different steps were as follows: the genes coding for the 2 variable regions of D7C2 cell line were inserted separately into the plasmids carrying a genetic cassette coding for the constant light chain and a cassette coding the constant heavy chain of human IgG1. The resulting fused genes are controlled by promoters P10 and Polyedrine respectively. A baculovirus light chain was obtained after transfection of insect cells with the plasmid light chain. the double recombinant baculovirus was then obtained after co-transfection and recombination between the light chain baculovirus genome and the heavy chain plasmid. Ig expression was studies by intra- and extra-cellular protein analysis after infection of SF9 insect cells. An evaluation of the biological activity of this recombinant antibody was made: (1) by an agglutination test in tubes, mixing the supernatant insect cell culture (30 µg/ml) and different papain-treated RBC. The test showed agglutination on all Rh^+ RBC. Moreover, the study of 1000 blood donors did not show any discrepancy when tested in parallel with other blood group reagents; (2) ADCC test, in which the effector cells (lymphocytes), the target cells (^{51}Cr-labelled RBC), and the recombinant anti-Rh (5 µg/ml) showed a specific lysis of 93%; commercial polyclonal Ig anti-Rh (µg/ml) tested in parallel showed a specific lysis of 76%. Moreover, we have performed several ***in vitro*** studies of this recombinant antibody and have shown:

(i) the recombinant antibody was unable to bind complement; (ii) a mixture of 90% Rh^- and 10% Rh^+ revealed by flow cytometry that all Rh^+ cells were labelled by antibody.

The results are comparable to those obtained with polyclonal anti-Rhesus antibody tests in parallel. Animal and human clinical trials of this recombinant antibody are in progress.

Paratopic specificity of two human monoclonal immunoglobulins M expressing Y7 idiotype

Lj. Dimitrijevic, M. Radulovic, B. Ciric, A.Inic, and S. Apostolski

Immunology Research Center "Branislav Jankovic", Beograd, YUGOSLAVIA

We have already characterized Y7 idiotype as natural idiotype by showing its expression on IgM molecules derived form cord sera. The aim of this study was to investigate the binding properties of two human monoclonal IgM antibodies isolated from the sera of patients with Waldenstrom macroglobulinaemia, which express Y7 idiotype. In order to estimate the binding repertoire of these two IgM molecules (IgM DJ and IgM RD) we tested the isolated IgM molecules and their F(ab)2 and Fab fragments over the panel of 23 different protein and non-protein antigens, nine different bacterial strains and cell membranes from neuronal and epithelial tissues. These tests were performed by means of direct and competitive ELISA, immunoblot and immunofluorescence assays. Both antibodies reacted with ssDNA, oligonucleotide fragments, and lactobacteria. IgM DJ showed reactivity with myelin associated glycoprotein (MAG) and blood vessel endothelial cells. Binding for DNA oligonucleotide fragments and lactobacteria was interrupted up to 70% with 0.5 M NaCl, which indicated that these interactions were of low affinity. Avidity dependent binding was also evidenced by use of F(ab)2 fragments. Determined characteristics such as polyreactivity which included autoantigens, low affinity and avidity dependent binding, qualified these two monoclonal IgM as natural autoantibodies. Expression of natural idiotype Y7 on two immunoglobulins M,,which possessed natural autoantibody properties, indicated the connection of natural autoantibody specificity and the natural idiotype expression and suggested the involvement of network interactions in selection of malignant B cell clones.

Effects of recombinant IL-2 and T-cell subsets on peripheral B cells in patients with myasthenia gravis

Jian Zhang*, Wenbin Zhou, and Honglin Wang

Institute for Neurological Diseases, Xiangya Hospital, Hunan Medical University, People's Republic of CHINA. [**Present address: John P. Robarts Research Institute, London, Ont. CANADA*]

Myasthenia gravis (MG) is an autoimmune disease of neuromuscular transmission characterized by the presence of autoantibodies directed against acetylcholine receptor (AChR). The abnormalities of regulation in cellular immunity leading to a large amount of AChR antibody (AChR-Ab) production by B cells in MG patients may be responsible for its immunopathogenesis. To further address the regulation of AChR-Ab production in MG patients, the proliferation and differentiation of peripheral B cells were studied in 12 MG patients and 10 control subjects. In particular, we observed the effects of recombinant IL-2 (rIL-2) and T-cell subsets on proliferation and differentiation of B cells from MG patients. Our results showed that the capacity of peripheral B cell proliferation in MG was higher than in control subjects and was enhanced by rIL-2 and $CD4^+$ T cells compared with the unfractionated T cells. There was no significant increase in AChR-Ab production by removing $CD8^+$ T cells compared with the unfractionated T cells. Furthermore, the production of AChR-Ab was decreased when rIL-2 was added in the presence of $CD8^+$ T cells. These findings suggest that B cells from the peripheral blood in MG patients were hyperactive and that there was some defect in $CD8^+$ T cells which resulted in an imbalance in ratio of $CD4^+$ to $CD8^+$ T cells. Most likely, rIL-2 suppresses the production of AChR-Ab of peripheral B cells in MG patients by increasing the suppressor activity of $CD8^+$ T cells.

Anti-gp210 antibodies in sera of patients with primary biliary cirrhosis: identification of a 64 kD fragment of gp210 as a major epitope

Jozefa Wesierska-Gadek, Eva Hitchman* and Edward Penner*
*Institute of Tumorbiology-Cancer Research, and *4th Department of Internal Medicine, University of Vienna, Vienna, AUSTRIA*

Patients with primary biliary cirrhosis (PBC) frequently have autoantibodies directed to gp210, an integral glycoprotein of the nuclear pores. This protein contains a large glycosylated lumenal domain, a single hydrophobic transmembrane segment and a short cytoplasmic tail. It has been previously shown that autoantibodies from PBC patients excludively react with the cytoplasmic tail when recombinant rat gp210 protein expressed in *E. coli* was used as antigen. Using human gp210 isolated from HeLa cells we found the lumenal domain as a major target. The aim of this study was to further characterize the dominant autoepitopes of gp210. Sera from 88 patients with autoimmune liver disease and 20 controls were used. gp210 protein was digested with papain or endoglycosidase H and then subjected to immunoblotting. Autoantibodies against gp210 were detected in 12 of 43 (28%) PBC patients, but in none of the autoimmune hepatitis and control sera. Four of 12 (33%) anti-gp210 positive sera reacted with a fragment consisting of the cytoplasmic tail and 8 (66%) sera targetted an epitope located within the large lumenal domain. Furthermore, our data show that antigenic determinant is restricted to the 64 kD glycosylated amino-terminal fragment and that carbohydrate residues are an essential part of this novel epitope. Moreover, we demonstrate that anti-gp210 positive PBC sera recognize two glycosylated gp210 fragments of about 60 kD that are generated by endogenous nuclear proteases. We suggest that antigens possessing both epitopes, namely: the glycosylated lumenal domain and the cytoplasmic tail should be used for screening tests in order to detect all sera with anti-gp210 specificity. Since gp210 is easily degraded by endogenous proteases, the antigen should be carefully prepared to avoid its undesired degradation.

Effect of *Tripterygium wilfordii* Hook on anti-dsDNA secretion of PBMC from systemic lupis erythematosus patients

Qin Wanzhang and Xia Jinhua
Dept of Dermatology, Shanghai Medical Univ., Zhongshan Hospital, Shanghai, People's Republic of CHINA

The amount of total IgG and specific IgG (anti-dsDNA) in sera and the culture supernatants of PBMC spontaneously secreted in six patients with active SLE. It was shown that both IgG and anti-dsDNA of active SLE pateints were significantly higher than that of inactive subjects, the latter was markedly elevated compared to that of normal donors and the level of anti-dsDNA secretion *in vitro* was more closely correlated with that of sera. We found that native DNA (nDNA) was able to elevate anti-dsDNA antibody production of SLE PBMC, but no obvious effects on total IgG level and did not induce normal PBMC to secret anti-dsDNA antibodies. It was indicated that there are a lot of active B lymphocytes expressed by IL-2 receptor in inactive patients' blood as well as that of active patients. We did not find the role of exogenous rIL-6 on anti-dsDNA antibody secretion. Using the model which DNA antigen inducing anti-dsDNA antibody production by SLE patients' PBMC, we have studied the therapeutic mechanism on SLE with *Tripterygium wilfordii* Hook (TWH) and found three TWH monomers used in this paper are able to suppress antibody secretion and their effects are more significant than methylprednisolone.

Antibodies to human heat shock protein in patients with schizophrenia and major depression

P. Sirota, D. Mazeh and A. Novogrotsky*

*Abarbanel Mental Health Center and Tel Aviv University, and *Felsenstien Institute for Medical Research and Tel Aviv University, Ramat Aviv, ISRAEL*

Different immunological abnormalities were reported in schizophrenia and major depression. Heat shock proteins are produced in every cell type in reaction to different stressors. They are found in cells in normal conditions in minor quantities. In autoimmune diseases they were found in large quantities. Kilidries *et al.* reported an increase of hsp60 antibodies in the serum of patients with schizophrenia. The serum of 26 patients with schizophrenia, 8 with major depression and 22 normal subjects, were tested for the presence of hsp antibodies. The serum samples were tested for antibody binding to protein extracts of IMR-32 neuroblastoma cell line on Western blots. In Western blots IgG in the serum of all patients but one, and of normal subjects, reacted with a protein of 60 kD. The intensity of each band in the 60 kD region was quantified in a scale of four groups. No significant differences were found among the three groups. On the other hand, IgG of 8 patients with schizophrenia (30.71%) formed a band in the region of 85 kD. This band was not formed with the serum of other groups. hsp60 kD is an antigen of many pathogens and antibodies against it can be a result of an infection and may thus not be a good indicator of an autoimmune process. The presence of antibodies against hsp90 kD might be a much more specific indicator.

Auroantibodies to tyrosinase: crossroad between vitiligo and melanoma

O. Merimsky, Y. Shoenfeld*, E. Baharav, R. Tsigelman and P. Fishman

*Lab. Clinical & Tumor Immunol., Felsenstein Research Institute, and *Dept of Internal Medicine "B", Sheba University, ISRAEL*

Tyrosinase is an enzyme which participates in the process of melanin production in melanocytes. Recently we defined tyrosinase as a novel autoantigen in vitiligo - an autoimmune disorder manifested by the presence of white patches on the skin resulting from destruction of melanocytes by the immune system. Antibodies against tyrosinase were detected in the sera of patients with melanoma associated with white patches on their skin. These white patches imply for better prognosis and the presence of anti-tyrosinase antibodies in these patients may be regarded as an additional parameter of disease state. In the present study two beneficial clinical applications of anti-tyrosinase antibodies were evaluated.
The first entailed anti-tyrosinase antibodies as a marker for monitoring and follow-up of patients with melanoma treated by immunotherapy. Seven patients with metastatic melanoma were treated by vaccination with anti-idiotypic antibodies mimicking the high molecular weight melanoma associated antigen. The titer of anti-tyrosinase antibodies increased following the vaccination and then decreased most probably due to absorption to melanoma cells and melanocytes. The development of anti-tyrosinase antibodies in response to vaccination by another antigen may be explained by induction of non-specific polyclonal B-lymphocyte activation. The second employed therapeutic application of anti-tyrosinase antibodies. We have shown that C57BL/6J mice immunized with tyrosinase generated a high titer of anti-tyrosinase antibodies and following the inoculation of melanoma cells developed lower number of lung metastaces compared to an unvaccinated control group.

Development of anti-complement antibodies to treat both chronic and acute inflammation

S. Squinto, Y. Wang, S. Rollins, E. Mueller, M. Evans, S. Nye, T. Thomas, J. Wilkins and L. Matis
Alexion Pharmaceuticals Inc., New Haven, CT, USA

Activated components of the complement system play an important role in mediating inflammation in a large number of both immunologically-induced disease states such as immune complex vasculitis, glomerulonephritis, and joint inflammation, as well as in non-immunological conditions such as ischemia/reperfusion injuries. Thus, soluble complement inhibitor drugs would be expected to have considerable therapeutic potential as anti-inflammatory agents. To exploit this potential, we have developed for clinical use a potent blocking MAb against human complement component C5. Complement inhibition at C5 prevents the generation of complement components C5a and C5b-9 (membrane attack complex - MAC). By doing so, this complement inhibitory antibody prevents the potent anaphylatoxic and chemotactic activity of C5a as well as the pro-inflammatory and cell lytic activity of MAC. Importantly, the anti-C5 antibody inhibits both the classical and alternative complement pathways. Furthermore, the anti-inflammatory activity of the anti-C5 MAb is achieved without blocking the generation of C3b. This complement inhibitory strategy, therefore, preserves the pathogen opsonization and immune complex clearance activity associated with C3b. Therefore, MAbs that block complement at C5 provide for maximum anti-inflammatory potential without seriously immuno-compromising the patient. This safety feature would be critical in the treatment of chronic inflammation. Our anti-C5 MAb has proven to be highly effective in blocking inflammation and disease when tested in murine models of both glomerulonephritis as well as collagen II-induced arthritis. Additionally, an engineered scFv form of the anti-C5 MAb has also been proven to be highly effective at inhibiting the inflammatory cascade initiated during cardiopulmonary bypass, circulating whole human blood through a CPB circuit. Our studies indicate, therefore, that anti-C5 MAbs have significant clinical promise in the treatment of immune complex disease as well as vascular inflammatory disease.