A block in the expression of MHC class II genes as the molecular basis for congenital immunodeficiency ('bare lymphocyte syndrome')

C. de Préval, M. Loche, B. Grospierre, C. Griselli, M. Hadam and B. Mach
Department of Microbiology, University of Geneva, CH-1211 Geneva, Hôpital des Enfants Malades, Paris, France, and University of Hanover, FRG

The human class II (HLA-D) genes are a multigene family whose products play a central role in the immune response. A type of congenital immunodeficiency is characterized by patients who do not possess class II histocompatiblity antigens on the surface of their immunocompetent cells. We have analyzed the class II gene expression in lymphocytes of such patients. The following observations were made:
1) The defect is due to a block in gene expression (absence of mRNA). 2) All 12 class II genes are silent. 3) Class II genes themselves are not mutated as judged by family genetics and occasional reversion of cultured lymphocytes to class II positive cells. 4) Expression of HLA-DR associated invariant chain gene is not affected. 5) The defect is found in all immunocompetent cell tested. 6) Immune interferon does not induce class II gene expression.
We conclude that the molecular defect concerns a class II-regulatory gene, located outside of the MHC which could be a target for the effect of γ interferon.

Symptomatic *Toxoplasma gondii* infection in humans is associated with imbalance of immunoregulatory antigen-specific T lymphocytes

I. Sklenar, T. C. Jones and P. Erb
Institut für Mikrobiologie und Hygiene, Universität Basel, CH-4003 Basel, and Cornell Medical College, New York, USA

Infection with the protozoan *Toxoplasma gondii* causes no illness in most people. In approximately one out of five infected individuals malaise, fever and lymphadenopathy are recorded. In an effort to identify factors which separate those people who experience illness from those who do not, we examined peripheral blood T cell subsets and toxoplasma antigen-induced T cell subsets during toxoplasmosis. A total of 19 individuals were studied. Using flow cytometric analysis patients with recent symptomatic infection had significantly higher absolute numbers of T8+ cells (suppressor/cytotoxic) and lower T4/T8 (helper/suppressor) cell ratios than patients with chronic asymptomatic infection and not infected individuals. In addition, toxoplasma antigen-induced long term cultures showed prevalence of T cells with suppressor markers (T8, TQ1) and less cells with helper marker (T4) in symptomatic patients. One patient with predominantly T4+ cells in cultures had extremely high serum toxo-antibody titers. In addition, the acute symptomatic infection was accompanied by elevated numbers of monocyte/macrophages (Leu M3 +) and NK/K cells (Leu 7+). These data suggest that in patients prone to symptomatic infection toxoplasma is effective in inducing immunoregulatory T cell subsets characterized by markers and function as suppressor cells.

Bivalent vaccine strains of *Salmonella typhi* and *Salmonella typhimurium*

E. Fürer, W. Dallas, S.J. Cryz Jr, and R. Germanier
Swiss Serum and Vaccine Institute, CH-3000 Bern, and Burroughs Wellcome, Research Triangle Park, North Carolina, USA

The epimeraseless mutants, *S. typhi* Ty21a and *S. typhimurium* LT_2MlC, were transformed by a plasmid containing the cistron coding for the B subunit (LT-B) of the heat-labile toxin (LT) of *Escherichia coli*. LT-B was synthesized and secreted by both strains when grown in liquid culture. Immunization of mice with these strains evoked both an anti-bacterial and anti-LT immune response. Introduction of the plasmid coding for LT-B into *S. typhimurium* LT_2MIC did not diminish its immunizing capacity against a virulent strain of *S. typhimurium* in mice. These strains provide the potential to vaccinate against both the vector strain and diarrheal disease mediated by LT-related toxins.

Expression and production in *E. coli* of merozoite stage-specific polypeptide antigens from *Plasmodium falciparum*

M. McGarvey, L. Perrin and B. Mach
Department of Microbiology and Department of Medicine, University of Geneva Medical School, CH-1211 Geneva

Certain proteins, synthesized late in the asexual blood cycle of *P. falciparum*, have been shown to be able to confer some degree of protective immunity against malaria parasite infections.
Stage-specific cDNAs were identified by differential hybridization, by screening a *P. falciparum* blood stage cDNA library with probes made from either total ring stage (early) mRNA or total schizont-merozoite stage (late) mRNA.
From a library of 10,000 clones, 150 schizont-merozoite stage-specific cDNAs were identified and these were shown to correspond to 12 different genes. These cDNAs were expressed in *E. coli* using the inducible plasmid expression vector pP131A. Hybrid fusion proteins, which contained both a portion of a vector encoded protein and the expressed portion of the parasite cDNA, were synthesized at levels of up to a few percent of the total *E. coli* protein.
Protein immunoblots and immunoprecipitation experiments confirmed that some of these cDNAs coded for proteins which cross-reacted immunologically with known protective antigens (e.g. the 200 K mol. wt merozoite protective antigen). Examination of the DNA sequences of some of these cDNA clones has shown that they have the unusual feature of containing regions of repeated units of amino acids (e.g. examples of repeat units of 4, 5 and 6 amino acids in length have been found). These unusual protein structures have been implicated as having an important role in the interaction of the parasite with its host.

***Pseudomonas aeruginosa* polysaccharide-tetanus toxin conjugate vaccine**

S.J. Cryz, E. Fürer and R. Germanier
Swiss Serum and Vaccine Institute, CH-3000 Bern

Cyanogen bromide-activated polysaccharide (PS) from *P. aeruginosa* PA220 lipopolysaccharide (LPS) was linked to purified tetanus toxoid (TTXD) by use of adipic acid dihydrazide as a spacer molecule. Conjugates were composed of PS and TTXD at ratios of 1:1 to 2:1 and possessed a molecular weight greater than 350,000. Conjugates were nontoxic, nonpyrogenic and highly immunogenic in rabbits and mice. Anti-PS-TTXD antibody, elicited either in response to active vaccination, or passively transferred, were highly protective against fatal experimental *P. aeruginosa* PA220 burn wound sepsis.

Results of a vaccination campaign against hepatitis B virus in a psychiatric clinic

F. Burkhardt and D. Jachertz
Institut für Hygiene und Medizinische Mikrobiologie der Universität Bern, CH-3010 Bern

In a psychiatric clinic with a known frequent occurrence of sporadic hepatitis B infections a campaign was undertaken to immunize 303 patients and 428 personnel with a killed vaccine (Hévac B). All vaccines had been tested previously for HBV-markers and individuals positive for antigens or antibodies excluded from the trial. 1 month after the last injection, 381 out of 428 personnel (89.2%) had anti-HBs antibodies, whereas of the

303 patients, only 148 (48.8%) reacted to the vaccine. The influence of administered drugs on the weak response within the patient's group is discussed.

Two different configurations of rabies glycoproteins

R. Glück, U. Kihm, A. Wegmann and R. Germanier
Department of Virology, Swiss Serum and Vaccine Institute, CH-3000 Bern

In an attempt to develop a potent purified duck embryo rabies vaccine, the physical state of the rabies glycoprotein (GP) antigen has been investigated. Viral antigens derived from embryonated duck eggs were analyzed by density gradient centrifugation, ELISA and determination of infectivity. The results revealed that GP which banded at a density of 1.16 g/cm^3 was an integral component of the intact virion. At a density of 1.19 g/cm^3 high levels of GP were still noted, whereas the infectivity titer had decreased by three logs. Presumably GP in this band was present in form of aggregates with cellular lipids. The two fractions were treated with β-propiolactone and varied in regard to mouse-protective properties and to the antibody response of dogs and humans. These findings suggest that the rabies GP is highly immunogenic when present at the surface of the virus particle. The low immunogenicity of GP in spontaneously formed aggregates may be due to the production of weakly immunogenic complexes with cellular lipids and/or to the presence of soluble GP as recently described by Dietzschold et al. (1983).

Specific immunoglobulin responses in primary Q fever

O. Péter, G. Dupuis and W. Burgdorfer
Division of Clinical Microbiology, Institut Central des Hôpitaux Valaisans, CH-1950 Sion, and Rocky Mountain Laboratories, Hamilton, MT, USA

A knowledge of the different antibodies appearing in acute Q fever as well as their development during the course of this illness are invaluable to the clinicians when interpreting the results of serological analysis. The present study shows the development, during the year following a primary infection, of the antibodies to *Coxiella burnetii* phase I and II detected by complement fixation and immunofluorescence (IgG, IgM). We have evaluated 683 sera taken from 191 patients who had contracted acute Q fever in the autumn of 1983 in Switzerland, during an epidemic in which 415 cases were serologically confirmed.
As a general rule, it was found using complement fixation that the antibodies to *C. burnetii* phase II stay raised during the year following acute Q fever, whilst the antibodies to *C. burnetii* phase I are hardly discernable. By immunofluorescence, IgG anti-phase I and II follow the same pattern, but the sensitivity is higher. In addition, IgM anti-phase I and II appear earlier but only remain for 10–12 weeks on average. Anti-phase II antibodies are generally much higher than anti-phase I antibodies during this period.

Antigen-free vaccination, results of placebo-controlled studies

D. Jachertz
Institut für Hygiene und Medizinische Mikrobiologie, CH-3010 Bern

In contrast to the well known methods of active immunization, antigen-free vaccination consists in transfer of immunologically relevant, information as informative RNA (i-RNA), which is free from antigen. The mode of action of the i-RNA in recipient cells is described.
Placebo-controlled double-blind tests have been performed using i-RNS against influenza virus A-equine-Miami 942, herpes simplex virus type 1 and varicella zoster virus. The significance of sero conversion against influenza-virus-A-equine-Miami 942, was $p = < 0.01$. In patients suffering from herpes simplex virus infection application of i-RNA reduced the frequence of recidives per year from 7.6 (12 months before i-RNA) to 0.8 (12 months after i-RNA). Compared to the placebo group (frequency of recidives not changed) the significance of this reduction is $p = < 0.05$. In the placebo controlled study in patients suffering from herpes zoster the difference between the i-RNA treated group and the placebo group concerning 11 different parameters was also significant with a $p = 0.0007$.

LIA: a procedure employing multiantigen nitrocellulose strips for the simultaneous detection of IgG or IgM antibodies in serum

J. A. Wilhelm, L. Matter, W. Wunderli, F. Roth and K. Schopfer
Institute of Clinical Microbiology and Immunology, CH-9000 St. Gallen

The use of multi-dotted nitrocellulose strips (Hawkes et al., Analyt. Biochem. *119* (1982) 142) has been adapted for semi-automated serum antibody detection for diagnostic purposes. Antigens are applied in parallel lines on a nitrocellulose sheet together with controls and standards. The strips are cut and incubated with serum, HRP-conjugated anti-human Ig (G or M) and finally H_2O_2 with chloronapthol as indicator. The dried strips are scanned in a densitometer. A comparison with classical methods (CF, IF, EIA or ELA) showed a good correlation. A negative: positive cutoff value was thus determined for each antigen.
IgG antibodies to toxoplasma, HSV, VZV, CMV and mumps virus is routinely assessed by this method, as well as IgM to CMV, VZV and HSV antigens. The inclusion of IgG on the IgM strip allows the detection of rheumatoid factor, which can be absorbed with IgG-latex. Results are available within 1 day for 5 (or more) antigens using 10 µl of serum (or antibody containing secretions).
Details of the test procedure, comparisons with other tests, correlations with clinical situations, and the advantages and problems will be discussed. We call the procedure 'LIA': a descriptive term denoting Line Immuno Assay.

Rapid Diagnostic Methods in Microbiology

Detection of human cytomegalovirus in clinical specimens through nucleic acid hybridization

P. Gowland and W. Wunderli
Institute of Clinical Microbiology and Immunology, CH-9000 St. Gallen

The diagnosis of human cytomegalovirus (HCMV) infection presently involves either a titer rise of IgG antibodies to HCMV in serum, the detection of IgM antibodies to HCMV, or isolation of the virus on human fibroblast cell culture. Due to the slow growth of fresh clinical isolates, many cultures take up to 6 weeks before being identified as positive.
We have established a DNA-DNA spot hybridization system for the detection of HCMV in clinical samples (e.g. urine). Viral DNA was isolated from either fresh clinical samples or from the medium of an infected fibroblast culture and bound denatured to nitrocellulose filters. The filters were subsequently hybridized with ^{32}P-labelled cloned Hind III fragments of HCMV strain AD 169 (kindly provided by H. Gadler, Stockholm, Sweden). After washing specific hybridization was detected by autoradiography. The assay can detect less than 1 ng of viral DNA within 3–4 days.