Scientifically Speaking

Old Turks and Young Turks

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Washington, DC—In the early part of this century merit in clinical research in the United States was most commonly recognised by election to the Association of American Physicians. The organisation's limited membership of a few hundred was ample to accommodate the worthy investigators of that time. Almost by definition, members of the AAP had already seen their most imaginatively productive years and now were heavily occupied in guiding younger researchers. The younger researchers, however, wanted an organisational forum for their best work while they were still doing it, and shortly before the first world war they formed the American Society for Clinical Investigation. One provision of the ASCI constitution was that a member became emeritus at the age of 45; they quite quickly became nicknamed Young Turks, and members of the Association of American Physicians were Old Turks. To this day in American medical research circles one worker will ask another, "You going to the Turks' meetings?" and never really know or recall how that particular collective noun came about.

The Turks' meetings are an annual feature of late spring. An even younger Turks organisation has been part of the festivities since the second world war—the American Federation for Clinical Research, whose members become "senior" at 41 and lose their floor privileges. The three groups hold their plenary sessions in a tight sequence of half days, attracting thousands and thousands of doctors and hangers-on to a breathlessly paced series of research presentations (10 minutes each). This spring the Turks descended on a major Washington convention hotel with a programme which, while heavy as always with technical niceties, also maintained the Turks' reputation as a principal weathervane for the directions being taken by university-based biomedical research in North America.

Brains and genes

Two of the most frenetically investigated topics in molecular biology—the brain's own opiate-like substances and the genetic manipulation of recombinant DNA techniques—were fused into a single accomplishment in a report from a group working at the University of California, San Francisco. They put together pieces of mostly natural DNA to form a sequence of genes that inspired bacteria to produce β -endorphin, the opiate-like peptide. As John D Baxter explained to the Association of American Physicians, the tricky parts of such a procedure

have to do with the circumstances that the genes going into the bacterial plasmid are not tidily segregated to produce only β -endorphin; nor do they have every last detail for the desired peptide. A few genetic fillips have to be given going in, and some careful separation accomplished coming out.

The gene sequences that provided the raw material came from the blueprint for a precursor protein that has amino-acids—overlapping—not only for β -endorphin, but also mouse melanocyte-stimulating hormone and adrenocorticotrophic hormone. That DNA, previously cloned, lacked a codon for one amino-acid of β -endorphin—the C-terminal glutamine—and a "stop" codon. Chemical synthesis filled in the missing necessities, and the experimenters went on to attach their gene fragment to a bacterial plasmid that contained the gene for β -galactosidase. They hoped that the bacteria taking up those plasmids would synthesise a hybrid protein that would contain, in some recognisable order, the amino-acids of β -galactosidase, β -melanocyte-stimulating hormone, and β -endorphin. Indeed, the bacteria did. The experimenters worked out a way to get the β -endorphin out of the hybrid protein by letting trypsin attack only certain of the residues vulnerable to it. The resulting product has the same size as a naturally occurring β -endorphin, competes for binding at opiate receptor sites in the rat brain with both an agonist and an antagonist, and acts like an opiate in blocking the stimulation by prostaglandin E_1 of cyclic adenosine monophosphate production of neuroblastoma-glioma cells.

What the experimenters have is mouse β -endorphin, which differs from the human variety by only two of 31 amino-acids and seems to have the same biological activity. Dr Baxter said that it would not take much chemical manipulation to make the substance identical to the human type, if that proved necessary. Although the experimenters are mainly interested in gene theory and characterisation, the practical application of this particular procedure has a big potential. At the very least, it is a cheap way to make β -endorphin: each bacterium produced about 80 000 molecules of it. Authors of the report were John Shine, now with the Australian National University Research School of Biological Sciences, Canberra; James L Roberts, now at Columbia University; and Ivy Fettes, Nancy C Lan, and Dr Baxter, all at the University of California, San Francisco.

We're in real trouble if legionnaires' disease ever becomes such a widespread threat as to call for a vaccine, according to a report presented to the American Society for Clinical Investigation by two investigators at the Rockefeller University in New York. The problem is that the pathogen grows inside human monocytes, sheltered from antibodies to it and subverting the mechanisms of the type of cell that would be expected to kill it. Marcus A Horwitz and Samuel C Silverstein had employed robustly virulent *Legionella pneumophila* Philadelphia 1 bacteria in incubation in a variety of circumstances. Only in the presence of whole monocytes did the bacteria multiply—not with human serum alone, or in monocyte-conditioned culture

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medium, or with lymphocytes, or with monocytes that had been disrupted by sonication.

At the peak of infection in a culture, electron microscopy showed monocytes packed with *Legionella pneumophila* organisms, almost all of them huddled in membrane-bound cytoplasmic vacuoles studded with structures that look like host-cell ribosomes. Similar ribosome-studded vacuoles have also been seen in white blood cells from the lungs of patients with legionnaires' disease. Antibodies to the *Legionella pneumophila* organism, taken from a survivor from legionnaires' disease, did not incite either monocytes or polymorphonuclear leucocytes to kill the bacteria. The Rockefeller investigators surmised that, if there is an effective natural defence against the bacterium, cell-mediated immunity would be required to do the job, not vaccine-induced antibodies. *Legionella pneumophila* appears to join a hearteningly small category of pathogens that parasitise monocytes in man. Others in the club are the agents of tuberculosis and leprosy.

Researchers at work

An immunological cause of human infertility, often postulated but seldom probed, was pinned down in a report to the American Society for Clinical Investigation by investigators at the University of Pennsylvania in Philadelphia. They had radio-tagged an antibody against immunoglobulin G (IgG) and went looking in the blood plasma of people with unexplained infertility for signs of IgG antisperm antibody. Among 107 couples with that complaint, the investigators identified 19% that had a significantly higher plasma content of antisperm antibody than was found in normally fertile control subjects. Men accounted for 7%, women for 9%, and both partners for 3%. A confounding factor is that almost half the subjects with antisperm antibody also had sperm-agglutinating activity in their plasma. But so did 10% of subjects without detectable antisperm antibody. The investigators speculate that agglutination is caused by another antibody or is not mediated by antibody.

Six of the infertile subjects with raised antisperm antibodies were monitored for changes. Two had spontaneous remissions in plasma antisperm antibody and conceived shortly thereafter. The four others were treated with corticosteroids and responded with a decline of antisperm antibody to normal concentrations.

According to the authors, G G Haas, B D Cines, and Alan D Schreiber, two of those four have now conceived.

The lurking presence of DNA and RNA sequences from hepatitis B virus may be identified in liver tumour cells, investigators at the Albert Einstein College of Medicine in New York told the American Society for Clinical Investigation. The import of the observation, David A Shafritz and associates said, is that actual virus production is no longer a requisite for following the supposed trail from hepatitis B virus infection to cirrhosis and thence to liver cancer. With some sharpening of their techniques, which employ molecular hybridisation and DNA recombination, it should be possible in future to determine from a needle biopsy specimen whether DNA exists as a leftover of a hepatitis B infection. Co-authors of the report were Prasanta Chakraborty, Daniel Shouval, and Nelson Ruiz-Opazo.

Monoclonal antibody techniques have produced a sleuthing substance whose differential binding to platelets can identify heterozygotes for hereditary thrombasthenia, Washington University investigators reported to the Society for Clinical Investigation. The condition, which is the most common of congenital platelet disorders, is marked by a deficiency of two glycoproteins in the platelet membrane. The St Louis experimenters had immunised mice with human platelets and fused their spleen cells to a mouse myeloma cell line so as to produce half a dozen different antiplatelet antibodies. Once the hybridoma lines were separated and cloned to produce an array of monoclonal antibodies, one, dubbed *Tab*, was found to be directed against the glycoprotein complex deficient in hereditary thrombasthenia but did not bind with anything else in sight, including lymphocytes or erythrocytes.

Tab has almost no binding affinity with the platelets of patients with thrombasthenia. Normal subjects bound almost 40 000 *Tab* molecules per platelet, which is probably the number of glycoprotein binding sites. Relatives of the patients, both known and presumed heterozygotes, bound an average of 24 500 *Tab* molecules per platelet. Rodger P McEver, who presented the report, said that the glycoprotein complex that grabs *Tab* is necessary for normal platelet aggregation and clot retraction, possibly because fibrinogen links to each of the glycoproteins in the normal platelet membrane. His co-authors were N L Baenziger and Philip W Majerus.

No reprints of this article will be available from the authors.

What treatment is advised for an individual bitten by a rabid dog?

The prevention of rabies developing in a previously unvaccinated person infected with the virus depends on the local treatment of any wound and the combined use of antirabies serum and rabies vaccine. Transmission of rabies virus is most likely when the skin is penetrated by the bite of a rabid animal but may also occur when rabies-infected saliva touches abraded skin or mucous membrane. Airborne infection is rare and has only been known to occur in bat caves and laboratories.

As an immediate first-aid measure the bite wound, scratch, or other possibly contaminated area should be washed thoroughly with soap and water. Then, under medical supervision, the wound should be repeatedly swabbed and flushed with a 20% soap solution and surgical treatment given as needed; the suturing of wounds should be avoided or delayed. Local procaine-type anaesthetics may be used. As part of the local treatment antirabies serum or human antirabies immunoglobulin should be infiltrated into the depth of the wound. Antitetanus and antibiotic treatment is given as indicated.

Antirabies serum or immunoglobulin is produced either in animals, usually horses, or from blood donated by people vaccinated against rabies. A single dose of animal antirabies serum (40 IU/kg of body weight) or human antirabies immunoglobulin (20 IU/kg of body weight) is given. Up to half should be infiltrated into the wound area; the remainder is given intramuscularly. To avoid an anaphylactic reaction tests for sensitivity should be performed before administering heterologous antirabies serum. About 40% of patients given heterologous serum develop serum sickness or rashes. Hypersensitivity reactions may be avoided by using human antirabies immuno-

globulin but because of its greater immunosuppressive effect give only 20 IU/kg, and only with vaccines of proved potency. Current vaccines used for postexposure treatment in man include those of nervous-tissue origin prepared from infected brain tissue of adult or very young animals, avian vaccines prepared in duck embryos, and cell culture vaccines prepared in human diploid or hamster kidney cell cultures. Vaccines used in man should be inactivated. The most potent rabies vaccine available is that produced commercially in France and Germany in human diploid cell cultures. Because of the superior potency the number of doses may be reduced. The schedule recommended is a single dose (1 ml) on days 0, 3, 7, 14, 30, and 90. Neurological complications have not been reported after its use, but local reactions, febrile influenza-like illness, rashes, and vomiting have occurred in some patients. No treatment failures have been reported. Less potent nervous-tissue and avian vaccines must be given in daily doses for 14-21 days, but neurological complications and treatment failures have followed their use. With all postexposure treatment schedules, booster doses of rabies vaccine should be given after the initial course to overcome any suppression of active immunisation by the use of serum and to maintain adequate levels of rabies antibody over a prolonged period. A person previously vaccinated against rabies with a vaccine known to be immunogenic and subsequently exposed to a rabid animal should receive 3-4 doses of a potent vaccine. They should not be given antirabies serum or human antirabies immunoglobulin.