In This Issue

Anthrax Lethal Toxin turns normal immune mechanisms against the host.

The anthrax bacillus produces a Lethal Toxin which acts on macrophages and appears to turn their antibacterial defense mechanisms against the host. Previous studies showed that low concentrations of the toxin cause macrophages to overproduce certain cytokines, inducing lethal shock. High toxin concentrations, however, cause the macrophages to undergo lysis. It has now been shown that this lysis results from overproduction by the macrophages of the reactive oxygen intermediates (ROIS), which are generated to kill invading bacteria. A model for Lethal Toxin pathogenicity can now be envisaged where, at the onset of anthrax, low doses of toxin initiate antibacterial ROI responses and expression of defense-related cytokines by macrophages. As toxin levels increase, high levels of ROIS are reached which lyse the host cells releasing the inflammatory cytokines and causing lethal shock symptoms. With respect to therapy, while antioxidants may be useful to protect the host cells from lysis, they may also compromise the ability of these cells to kill the bacteria.

High scrapie prion titers and infectivity in the absence of clinical symptoms.

Prions, the infectious agents causing scrapie and bovine spongiform encephalopathy in animals and Creutzfeldt-Jakob disease in man, appear to be modified forms of a normal host protein encoded by the PrP gene. It has been proposed that when prions (PrP^{Sc}) are introduced into a normal cell, they cause the conversion of a precursor, PrP^{C}, into PrP^{Sc}. Studies using transgenic mice carrying different numbers of copies of the PrP gene, and expressing PrP^{C} at different levels, have shown that susceptibility to disease correlates with PrP expression levels. These studies have now been extended to reveal that mice carrying a single copy of the PrP gene and low concentrations of PrP^{C} have levels of PrP^{Sc} and of scrapie infectivity very similar to congenic wild type animals early in the course of infection, but they show a much delayed onset of disease and slower progression to death. In consonance, while the development of neuropathological abnormalities characteristic of mouse scrapie is only marginally delayed in mice with a single copy of the PrP gene, the mice show no clinical symptoms for many months. These results suggest that an organism with low levels of PrP gene expression may carry high levels of PrP^{Sc} and scrapie infectivity, and the classical neuropathological abnormalities, without any clinical signs of infection.

Pentoxifylline, an inhibitor of the phosphorylating enzyme protein kinase, suppresses the human immunodeficiency virus type 1 replication and gene expression.

The expression of the human immunodeficiency virus type 1 (HIV-1) is regulated by both viral and cellular proteins which interact with specific sequences within the viral genome. One of the cellular proteins involved is NF-κB, present in most cells as an inactive complex that can be activated to promote gene transcription by agents such as cytokines and phorbol esters (PMA). Pentoxifylline is known to inhibit HIV-1 replication and gene expression. It has now been shown to act specifically by blocking the activation of NF-κB, a reaction catalyzed predominantly by the phosphorylating enzyme protein kinase C (PKC) when NF-κB activation is induced by either TNF-α or PMA. These findings strongly support the potential role of PKC inhibitors as anti-HIV-1 drugs. Given that PKC plays a key role in a number of vital signalling pathways in the cell, it is anticipated that high doses of PKC inhibitors would be cytotoxic. The effective concentration of PKC inhibitors can, however, be lowered if used in combination with other antiviral agents.

Reactive arthritis triggered by conserved bacterial antigens which are homologous to some human proteins.

Reactive arthritis describes a local inflammatory process mediated by T-cells. While this immune response is directed primarily towards bacterial antigens which are often present in inflamed joints, autoimmune responses to self-antigens have also been implicated. By analyzing the immunological activities of cells from ten patients

with reactive arthritis triggered by *Yersinia enterocolitica*, two distinct *Yersinia* proteins, the urease β-subunit and the 50S ribosomal protein L23, were identified to be immunodominant for the T-cell response. Amino acid sequences within the proteins were found to be conserved to varying degrees among diverse bacteria and to have homologous counterparts in some higher organisms, including man. The conservation of antigens capable of triggering the T-cell response involved in reactive arthritis could indeed explain not only why similar clinical manifestations are induced by different bacterial species but also suggest that the autoimmunity might result from homology between bacterial and human proteins.

Acquired thalassemia is frequent in patients with common variable immunodeficiency.

Common variable immunodeficiency (CVID) defines a heterogeneous group of primary immune system disorders. CVID patients are characterized by a marked deficiency of all immunoglobulin isotypes due to an arrest during the normal development of B-cells. Four out of six CVID patients examined have abnormal ratios of α and β globin chains of hemoglobin A, the hallmark of a particular class of anemias known as the thalassemias. While thalassemia can be inherited as a dominant trait due to mutations in the globin genes, the molecular nature of the acquired form of the disease is unknown. Analysis of one patient with CVID and acquired thalassemia suggests that myeloid and lymphoid cells arose from a single progenitor cell, which must have carried the primary defect observed in this patient's hematopoetic tissue. The implications are two-fold. First, thalassemia appears to be present in a significant proportion of patients with CVID. Secondly, while the precise molecular defect remains to be determined, a common mutation in a pluripotent stem cell is implicated in the acquired abnormality in both globin gene expression and B lymphocyte differentiation.

In monozygotic twins differences in X chromosome inactivation patterns are more pronounced in those with separate than with common placentas.

Despite their identical inheritance, subtle biologic differences have been observed between monozygotic (MZ) twins. It has been suggested that these may account for the discordant expression of certain diseases and phenotypes. The pattern of X chromosome inactivation, a trait which can be distinguished among individual females, was determined for 15 pairs of normal female MZ twins in whom the fetal placental anatomy was determined at birth. The results show that the X chromosome inactivation pattern in peripheral blood cells is more similar in monochorionic twins who share a common placenta, than in dichorionic twins with separate placentas. These results establish for the first time a relationship between a biologic trait and fetal placental anatomy in MZ twins. As discordant expression of autoimmune diseases is common in MZ twins, X chromosome inactivation patterns of female MZ twins with autoimmune disease was also evaluated. No association between X chromosome inactivation and expression of the disease was found, suggesting that the high discordant rate for autoimmune disease may be due to other factors.

Circulating cells with both leukocyte and fibroblast-like properties are involved in tissue repair.

The repair response to wounding involves a rapid influx of peripheral blood cells which act to prevent infection and to degrade damaged connective tissue components. At the same time, fibroblasts produce proteins which form the scar tissue. It is generally accepted that these fibroblasts arise from adjacent connective tissue. It has now been shown that peripheral blood contains appreciable numbers of circulating fibroblast-like cells which proliferate in regions of scar formation. These previously undescribed cells, which possess both fibroblast-specific protein markers and leukocyte-associated cell surface antigens, have been named 'fibrocytes'. The fibrocyte appears to be an important new player in the earliest phases of the physiological response to tissue injury. Fibrocyte entry into damaged sites may have relevance to diseases which involve impaired wound healing and poor scar tissue formation, such as ischemia or diabetic vasculopathy, as well as conditions characterized by excessive fibrosis, such as pulmonary and hepatic fibrosis or atherosclerosis.

Mutations causing Gaucher disease.

Gaucher disease is the most common glycolipid storage disorder and, while the majority of pa-

tients have mild clinical sysmptoms, there are severe cases with serious neurologic manifestations. Gaucher disease is inherited as an autosomal recessive trait and is due to mutations affecting the gene coding for glucocerebrosidase (glucosylceramidase) that cause a deficiency of the enzyme. There are still some individuals with the disease in whom no previously described mutations are identified in either one or both glucocerebrosidase alleles. The DNA from twenty-three such patients was examined and eight previously unidentified mutations that cause the disease have been characterized. Together with the other known mutations, they have been classified on the basis of the severity of the disease. The identification and classification of all glucocerebrosidase alleles causing disease will ultimately allow for more reliable genetic counselling of prospective parents.

Septic shock caused by bacterial endotoxin is mediated by a multistep signalling pathway leading to cytokine production.

The pathogenic processes by which microbial toxins elicit their adverse effects on hosts are extremely complex. Endotoxin or lipopolysaccharide (LPS), stimulates macrophages to synthesize various cytokines, the most important being tumor necrosis factor (TNF) which is a major mediator of septic shock. One step towards understanding the bacterial pathogenic process, is to elucidate how LPS transmits the signal to synthesize and release TNF. It has now been directly determined that LPS stimulates a multistep signalling pathway beginning with the activation of the proto-oncogene *ras*, which is followed by the activation of the protein kinase *raf-1* and the sequential activation of various extracellular signal-regulated kinases (MEKs and ERKs) and mitogen activated protein (MAP) kinases. Significantly, dominant inhibitors of *ras* and of *raf-1* specifically block LPS induced activation of TNF biosynthesis, suggesting that at least these two components of the 'MAP kinase pathway' are directly involved in LPS signalling. The identification of all the key players in the pathway responsible for transmitting the LPS signal to the level of the TNF gene should provide multiple points for inhibition, thus preventing cytokine release and induction of septic shock.

Inhibition of angiotensin II has deleterious effects in a mouse model of glomerulosclerosis.

Angiotensin converting enzyme inhibitory (ACEi) therapy delays the onset of renal failure in patients with diabetic nephropathy. In confirmation, treatment with either ACEi or angiotensin II receptor antagonists (Ang II RA) prevents or attenuates the development of glomerular lesions in a number of animal models. To further investigate the potential role of angiotensin II in the development and progression of glomerular lesions, ACEi and AngII RA were administered to transgenic mice with glomerulosclerosis induced by an overexpression of growth hormone. In this case, blockade of the renin angiotensin system resulted in a worsening of the glomerular lesions in the treated mice, apparently by upsetting the balance between synthesis and degradation of glomerular extracellular matrix components. The growth hormone-glomerulosclerosis mouse model suggests that in some forms of glomerulosclerosis the lesions develop independently of angiotensin II and that pharmacological inhibition of angiotensin II could be deleterious.