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0900 Apoptosis and Oncogenesis

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Apoptosis of mammalian cells represents the execution of a highly-conserved cell death pathway. Its consistent structural features reflect a common effector mechanism, usually triggered by activation of caspases – a family of around 15 proteases which cleave several cellular substrates at functionally critical sites. Caspase activation is itself regulated by signalling pathways, some linked directly to surface receptors (for example, TNFR1, Fas), others dependent on stimuli arising from injury affecting the cell membrane, mitochondria, nucleus and perhaps other organelles. The transduction of some but not all of these stimuli requires new transcription. Oncogenesis, in response to transforming viruses or other stimuli, is frequently if not invariably associated with selective loss of function of some of the pathways that converge on caspase activation, although loss of caspases themselves is probably not involved. One implication of this is that tumours constitutionally lose pathways that might otherwise have been activated in the course of therapeutic measures.

0945 Human papillomavirus type 16 stimulates cellular schizophrenia

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Human papillomaviruses (HPV) cause hyper-proliferation of stratified epithelium and with a subset of viruses this can result in malignant disease. The virus infects basal epithelial cells and when cells divide one of the daughter cells moves up through the epithelium and terminally differentiates. Since viral DNA replication occurs in the upper parts of the epithelium where terminal differentiation takes place, viral proteins must have activities, which stimulate cells into S-phase. This will ensure a plentiful supply of the cell's replicative machinery for viral DNA propagation and virion production. However, the complete viral cycle requires some level of differentiation, therefore the virus appears to be able to uncouple S-phase progression from cellular differentiation. I will discuss the activities of the HPV type 16 proteins, which are involved in S-phase progression.

1100 Identification of a macrophage host range determinant that promotes infected cell survival in the left variable region of the african swine fever virus (asfv) genome

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ASFV targets cells of the mononuclear-phagocytic system in swine and replicates efficiently in primary macrophage cell cultures. ASF viruses can, however, be adapted to grow in monkey cell lines. Characterization of two cell-culture-adapted viruses, MS16 and BA71V, revealed that neither virus replicated in macrophage cell cultures. Infection with these viruses resulted in early macrophage cell death which occurred prior to progeny production. Cosmid clones from a pathogenic ASFV isolate were used in marker rescue experiments to restore

MS16 and BA71V growth in macrophage cultures. A clone, representing an 11 kbp region at the left terminus of the genome completely restored growth for BA71V. Sequence analysis indicated that both BA71V and MS16 had significant deletions in this genomic region and that these deletions contained multiple members of multigene family 360 and 530. Deletion of this 11 kbp region from a pathogenic ASFV isolate, Pr4, markedly reduced viral growth in macrophage cell cultures. Our findings suggest that these novel genes perform an essential macrophage host range function(s) that involves promotion of infected cell survival.

1145 Chronic hepatitis B: new therapies based on understanding the viral kinetics and immunopathology

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1. Recovery during acute hepatitis B is the result of control of HBV replication by cytokines (interferon-gamma, TNF-alpha, and IL-12) secreted by lymphocytes sensitised to HBV and lysis of HBV infected cells by NK cells and cytotoxic T cells amplified by the CD4 helper T cell response. Genetic factors influence outcome.
2. Chronic hepatitis B is associated with a reduced cytotoxic T cell response, possibly due to deficient CD4 helper T cell function.
3. Mathematical modelling of chronic HBV infection indicates that, although the half-life of the virus is short (<1 day), the half-life of the infected cell is relatively long (10-100 days) and therefore viral suppressive therapy would need to be continued for 1-10 years before viral elimination occurred.
4. Monotherapy with viral suppressive drugs such as lamivudine, and famciclovir is initially successful but is constrained by emergence of drug resistant viruses. As with suppression of HIV, combination drug therapy may hold more promise. New compounds such as adefovir, drugs which are highly active against HBV, may be useful in this role.
5. Interferon alpha, when given for 3-6 months at a dosage of 3-10 megaunits thrice weekly, in patients with necro-inflammatory activity (elevated ALT), produces a sero-conversion hepatitis associated with HBe antigen/antibody conversion in 30-40% of cases. This sero-conversion hepatitis is thought to represent lysis of HBV infected hepatocytes by NK cells, CD8 cytotoxic lymphocytes amplified by CD4 helper T cells sensitised to HBe epitopes.
6. Interferon alpha, when given for 3-6 months at a dosage of 3-10 megaunits thrice weekly, in patients with no necro-inflammatory activity (normal ALT), rarely produces a sero-conversion hepatitis associated with HBe antigen/antibody conversion. These patients derive no significant benefit from interferon therapy.
7. Therapeutically induced HBe antigen/antibody sero-conversion associated hepatitis may theoretically be encouraged by administration of agents altering TH1/TH2 balance in favour of TH1 help which induces a cytotoxic T cell response. Agents such as IL12 or interferon alpha8 may induce such a change and could potentially be usefully combined with viral suppressive drug therapy encouraging lysis of HBV infected cells and preventing emergence of drug resistant variants. The induction of cytotoxic T cell activity with therapeutic vaccines such as preS1/S protein or cDNA expressing this protein may also be of theoretical value, combined with viral suppressive drugs.

1400 Molecular mechanisms of cytotoxic lymphocyte induced cell death
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Cytotoxic T lymphocytes (CTL) kill by two independent pathways, the release of granzymes and perforin stored in lysosome-like secretory granules and the engagement of the Fas ligand with Fas on target cells. CD8+ T cells kill tumors, virally infected or bacterially parasitized targets by the granule-based pathway, while Fas killing primarily mediates autoregulation of T cell proliferation by CD4+ T cells. Human CTL or NK cells express five granzymes (Gr) which are serine proteases that have diverse cleavage specificities. Mice made deficient in perforin or GrB (aspartic acid specificity) and GrA (tryptase-like specificity) are highly susceptible to non-cytopathic and some cytopathic viruses, while mice that are Fas deficient accumulate excessive numbers of T cells. Recent work has elucidated the molecular pathways for cell death by granzymes. GrB induces apoptosis following perforin-assisted entry into the cytoplasm by activating the cell death proteases (caspases). GrA, on the other hand, kills via a caspase-independent pathway. Both granzymes deregulate mitochondrial function, which contributes to cell death. GrB- but not GrA-induced apoptosis is highly suppressed by cellular and viral genes such as the Bcl-2 family, and the orthopoxviruses serpin proteinase inhibitors that also inhibit caspases. Thus, granzymes A and B appear to have independent cell death pathways, which may contribute to CTL efficiency in controlling viral spread.

Open papers

1445 Establishment of a mouse model for studying bovine Papillomavirus Type 2 (BPV2)
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Papillomaviruses are difficult to study because their infectious cycle is dependent upon the growth and differentiation of the keratinocyte. It has been shown that athymic mouse xenograft models are useful tools for passaging and propagating papillomaviruses *in vivo*. However one of the major problems of previously described xenograft models for human and rabbit viruses is the availability of the graft tissue and virus. We therefore established a simple mouse xenograft model by using readily available transplant tissue and virus. A wart from a Galloway calf was used for preparation of infectious virus that has been identified by partial sequencing as BPV2. Calf scrotal skin was inoculated with BPV2 before grafting it to the dorsum of severe combined immunodeficient (SCID) mice. 5 months after infection the induced tumours showed histological features of papillomavirus infections, including hyperkeratosis, acanthose and koilocytosis. *In situ* hybridisation using BPV2 probes demonstrated the presence of BPV2 DNA in infected transplants. The production of infectious viral particles in these induced tumours could be shown by focus-forming assays. This model should be useful for characterising antiviral compounds and understanding the regulation of papillomavirus infections

1500 The vaccinia virus B7R gene encodes a 18-kDa protein that is resident in the endoplasmic reticulum and contributes to virulence
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In vitro transcription and translation analysis showed the vaccinia virus B7R gene product was a 21-kDa protein that, in the presence of microsomes, was processed into an 18-kDa mature form. The 18-kDa form associated with the microsomal membranes and was within the lumen of the vesicle where it was inaccessible to exogenous protease or an antibody raised against the B7R C-terminus. Within VV-infected cells, the 18-kDa form of B7R was detected late during infection in the endoplasmic reticulum where it co-localised with protein disulphide isomerase. A virus deletion mutant lacking the B7R gene and a revertant virus were constructed. Compared to wild-type and revertant viruses, the deletion mutant replicated normally in cell culture and had unaltered virulence in a murine intranasal model of infection. However, the deletion mutant was attenuated in a murine intradermal model where it induced a smaller lesion than the control viruses.

1515 The topology and distribution of the vaccinia virus A36R protein
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Vaccinia virus (VV) gene A36R encodes a 45-50-kDa protein that is part of the extracellular enveloped virus (EEV) outer membrane. Here we have characterised the A36R protein by coupled *in vitro* transcription and translation, and confocal and immunoelectron microscopy. Translation of the A36R mRNA *in vitro* in the presence of canine pancreatic microsomes produced a polypeptide that was associated integrally with the membrane and was sensitive to digestion by exogenous protease. In contrast, alpha factor remained resistant to proteolytic digestion due to its presence within the lumen of the microsome. Immunofluorescent analysis of virus-infected cells demonstrated that the protein was not exposed to the antibody until the cell membrane was permeabilised. In contrast, the vaccinia virus B5R protein was exposed prior to membrane permeabilisation. Lastly, immunoelectron microscopy of infected cells using the MAbs directed against A36R protein showed that the protein was present on IEV but not IMV particles. Moreover, on CEV particles the A36R protein was associated exclusively on the side of the virion in contact with the cell surface.

1600 Localisation of vp73 on the African swine fever virus double membrane
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African swine fever virus (ASFV) is a large complex icosahedral virus. It is composed of several concentric layers, the outer most envelope is derived from the plasma membrane. The capsid layer contains the major structural protein, vp73, and inner membrane(s) derived via wrapping by the endoplasmic reticulum (ER)^{1,2,3,4}. There is an inner core shell and the nucleoprotein core.

The ER wrapping model predicts that there are two membranes in the capsid layer as do the morphology and permeabilisation experiments¹. Preliminary measurement data for membrane thickness in cryo-sections now also support these findings. Data includes mean \pm SD. n. the virus outer membrane was 8.22 nm ($+1.05$. 43). the virus inner membrane was 8.00

nm (± 1.25 , 41), virus factory membranes were 8.76 nm (± 1.35 , 40), the plasma membrane was 8.67 nm (± 1.25 , 16) and mitochondrial membranes were 8.55 nm (± 2.16 , 22).

Biochemical data has now shown that vp73 and the ER membranes are intimately linked^{2,3}. The distribution of vp73 on the double membrane was determined by immunolocalisation of two different vp73 epitopes (4H3 and 17LD3) on cryo-sections, data include total gold colloids and the percentage on the outside, inside and on the two viral membranes. The 4H3 conformational epitope distribution was - outside 220 (27.7%), inside 218 (27.5%) and on the membranes 355 (44.8%) on 139 virions. The 17LD3 non-conformational epitope distribution was - outside 362 (30.6%), inside 340 (28.8%) and on the membranes 479 (40.6%) on 133 virions.

These data support our previous work, independently indicating that the icosahedral capsid of ASFV consists of a double membrane, not a membrane and a protein layer, and that vp73 is located both on the inside and outside of the membrane pair. Together the accumulating information suggest that the icosahedral virion is formed by a wrapping mechanism involving the ER and may be driven by the incorporation of vp73 on to the surface of the ER as the faces form within the cytoplasm.

(1. Rouiller et al, 1998, *J. Virol.* 72:2373-2387; 2. Cobbold & Wileman 1996, *J. Virol.* 70:8382-8390; 3. Cobbold et al, 1998 *J. Virol.* 72:5215-5223; 4. Andres et al, 1998 *J. Virol.* 72:8988-9001)

1615 Cloning, sequencing and expression of the structural genes of viral haemorrhagic septicaemia, a fish rhabdovirus

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Viral haemorrhagic septicaemia virus (VHSV) is a fish rhabdovirus of the genus *novirhabdovirus* and the causative agent of viral haemorrhagic septicaemia (VHS), a disease which causes considerable losses in farmed trout throughout Europe. In addition to farmed salmonid fish species, the virus has also been isolated from a large number of non-salmonid species in the marine environment, prompting the suggestion that VHS may have been introduced into salmonid farming sites through the use of untreated trash fish as a feed supplement. However, challenge experiments suggest that the marine strains are of low virulence for the freshwater species.

We undertook the sequencing of the complete genome of VHSV isolates from marine and freshwater species to identify sequences involved in the determination of virulence. The complete nucleotide sequence of VHSV was determined and sequence analysis identified an overall difference of less than five percent between isolates at both the nucleotide and amino acid level. This data suggests that the freshwater and marine isolates are highly related genetically and that only a few amino acid substitutions are responsible for the virulence of VHSV. Identification of the key residues involved in virulence will require the use of reverse genetics and progress towards recovering infectious VHSV from a cDNA clone will be presented.

1630 Prolonged excretion of poliovirus by an immunodeficient patient

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WHO is on target to eradicate wild polioviruses. The successful eradication of poliovirus is possible because the virus does not persist in the human gut or the environment for longer than a few months. Exceptionally, immunodeficient patients have been known to excrete poliovirus for up to three years. These cases have largely come to light when the patients developed paralytic poliomyelitis.

We describe the case of an immunodeficient patient who has been excreting poliovirus for up to 15 years. The patient is asymptomatic, although the virus differs by approximately 10% from the original Sabin vaccine strain, which we believe was the original source of the infection. The virus contains genetic markers which are associated with increased neurovirulence and has been shown to be neurovirulent in animal tests. Such a prolonged excretion of a potentially virulent poliovirus will affect the vaccination strategies adopted following wild poliovirus eradication.

1645 A prospective clinical virological study of 17 HIV positive women before, during and after pregnancy including HIV RNA load in different compartments at caesarian section

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The aims of this prospective study were to measure HIV load, using different HIV RNA quantification assays, and CD4 counts in a cohort of HIV-1 infected women on antiretroviral therapy during and after pregnancy, the HIV load at caesarian section in various body compartments, and the HIV status of the baby on follow up.

Samples were collected for plasma HIV load and CD4 count monitoring during pregnancy, from the mother and baby at the time of surgery, and on subsequent follow up. Maternal samples were tested for HIV-1 RNA using the Quantiplex bDNA versions 2.0 or 3.0 (Chiron), Amplicor Monitor version 1.5 (Roche) and Nucleic Acid Sequence Based Amplification (NASBA QT, Organon-Technika) assays.

Ten of 17 women had discrepant plasma HIV load results when comparing the assays used in the study, including 3 with pretreatment plasma HIV load results of < 600 copies/ml using three or four assays. Cerebrospinal fluid and amniotic fluid samples collected at caesarean section from 6 women have been tested and the HIV RNA levels were below the limit of assay detection. HIV proviral DNA and HIV RNA have not been detected in plasma samples collected from any infants after up to 13 months post-delivery.

0900 Adenovirus transformation

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Abstract not available

0945 Inhibition of TRAIL-, Fas ligand-, and TNF-induced apoptosis by adenovirus proteins

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Tumor necrosis factor (TNF) "death receptor" family members include TNFR1, Fas/Apo1/CD95, TRAIL-R1/DR4, and TRAIL-R2/DR5. Following engagement with their ligands, TNF, Fas ligand, and TRAIL, respectively, these receptors mediate apoptosis via activation of caspases. The adenovirus (Ad) E3-RID, E3-14.7K, and E1B-19K proteins block apoptosis through these death receptors. RID causes Fas and TRAIL-R1 to be internalized into endosomes which are transported to lysosomes where the receptors are degraded. RID inhibits TRAIL-, Fas-, and TNF-induced release of cytochrome c and activation of Caspase 3. RID also stimulates the internalization of TNFR1, and it plays a role in down-regulating TRAIL-R2. Our results were obtained using virus mutants that lack RID and/or E3-14.7K and/or E1B-19K. Also used were Ad vectors that express all E3 proteins and no others, RID plus 14.7K, RID alone, or 14.7K alone. Dileucine and tyrosine-based motifs are important for RID function. TRAIL-R1 was down-regulated by representatives of ~50 diverse Ad serotypes. As shown by others, E3-14.7K and E1B-19K inhibit cellular proteins that function in apoptosis. Immune killer cells destroy targets, in part, through the TNF, Fas, and TRAIL pathways. Considering that Ad has three independent proteins that block these pathways, immune killer cells must be important in controlling Ad infections.

1100 EBV and apoptosis: viral mimicry of cellular pathways

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Epstein-Barr virus (EBV), a ubiquitous herpesvirus, is associated with the development of both lymphoid and epithelial malignancies. The expression of EBV genes that protect infected cells from apoptosis is important for both the latent and lytic life cycle of the virus and may contribute to the oncogenicity of EBV. Of particular interest is LMP1, a virus-encoded membrane protein, which is the major transforming gene of the virus. LMP1 has diverse phenotypic effects in cells resulting from the induction of various genes such as the anti-apoptotic Bcl-2 and A20 proteins and these effects may contribute to the transforming capacity of LMP1. Many of the effects of LMP1 are reproduced by activating CD40, a member of the TNF receptor family, and recent work has shown that LMP1 functions as a constitutively activated CD40 receptor by interacting with a common family of TNF

NF- κ B and of the stress-activated JNK and p38 kinases resulting in the up-regulation of specific genes. The ability of the NF- κ B pathway to mediate the transcription of anti-apoptotic genes and the role of the JNK/p38 pathway in both apoptosis and oncogenic transformation suggests that these effects are responsible for the pleiotropic consequences of LMP1 expression.

1145 KSHV/HHV-8: From cell biology to pathogenesis

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Kaposi's sarcoma-associated herpesvirus (KSHV or HHV-8) is implicated in the etiopathogenesis of Kaposi's sarcoma (KS), primary effusion lymphoma and a subtype of Castleman's disease. In all three these neoplasms, KSHV is latently present in the vast majority of tumour cells, suggesting that KSHV latent proteins directly induce the proliferation of these cells. Among the latently expressed proteins of KSHV are cellular cyclin and FLIP (FLICE inhibitory) homologues, and latent nuclear (LNA-1) and membrane (K15) proteins. The potential role of these proteins in interfering with the cell cycle or blocking apoptosis will be discussed.

In addition, KSHV encodes an array of other cellular homologues, although their expression appear to be restricted to lytic infection. Functions of the viral bcl-2, IL-6, IRFs (interferon regulatory proteins) and chemokines (vMIP I-III) will be discussed.

Kaposi's sarcoma lesions can resolve with partial restoration of the immune system, i.e. post-transplantation KS resolves when immunosuppression is stopped and AIDS-KS often improves with HAART. This suggests that cytotoxic T cell (CTL) responses against KSHV might be important in the pathogenesis of KS. The identification of CTLs against this virus will be discussed.

1400 Herpesvirus saimiri strategies for t cell stimulation and transformation

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Herpesvirus saimiri, a rhadinovirus and tumor virus of New World primates, transforms human T cells to stable growth in culture. The cells retain many essential functional features of native, mature, and activated T lymphocytes including the MHC-restricted antigen-specific reactivity. Only few virus genes are expressed from the stably persisting non-integrated episomes, and no virus particles are released. A series of cell-homologous virus genes, such as the viral homologs to IL-17 or to superantigens, are not required for transformation, but may be relevant for the apathogenic persistence in the natural host. The oncogene *StpC* and the neighboring gene *Tip*, whose product specifically interacts with the T-cellular tyrosine kinase Lck, are necessary for the transformation of T lymphocytes. The unique possibility to facilitate the amplification of functional T cells may form the basis for possible therapeutic applications. In macaques, autologous transformed T cells persisted over months after transfusion, and were clinically well tolerated without any signs of disease. In addition, herpesvirus saimiri can be utilized to efficiently express foreign genes in human T cells providing novel perspectives for the development of T cell mediated immunotherapy.

Open papers

1445 Caspase-dependent covalent modification of p27KIP1 during apoptosis in EBV-transformed lymphocytes

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p27KIP1 is a crucial component of the mammalian restriction point and its expression is tightly controlled. Transcriptional and translational control of p27KIP1 have been described and, more commonly, ubiquitin-mediated proteasomal degradation. A recent study demonstrated caspase cleavage of p27KIP1 during apoptosis in endothelial cells. We shall present data to show that p27KIP1 is covalently modified in lymphoid cell lines during apoptosis. The modification is observed in B- and T-lymphoid cells in response to a variety of agents, indicating that p27KIP1 modification is a general feature of apoptosis in lymphoid cells. Caspase inhibitors implicate the caspase cascade in the modification of p27KIP1. Immunoprecipitation of p27KIP1 followed by Western blotting reveal that Sumo is present in the modified form of p27KIP1. To our knowledge this is the first description of Sumoylation of p27KIP1 and we are currently investigating the role of this modification.

1500 Epstein-Barr virus (EBV) suppresses apoptosis at a G2/M checkpoint activated by genotoxins

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Several EBV-negative Burkitt lymphoma-derived cell lines (for example BL41 and Ramos) are extremely sensitive to genotoxic drugs despite being functionally null for the tumour suppressor p53. They rapidly undergo apoptosis, largely from G2/M of the cell cycle. Although the treated cells can pass through S phase they are unable to complete cell division, suggesting that a G2/M checkpoint is activated. BL41 cells latently infected with EBV are protected from both apoptosis and cell cycle arrest, allowing them to complete the division cycle. However, a comparison with EBV-immortalised B-LCLs (which have functional p53) showed that EBV does not block apoptosis *per se*, but rather abrogates the activation of, or signalling from the checkpoint in G2/M. Furthermore, analyses of BL41 and Ramos cells latently infected with P3HR1 mutant virus which only expresses a sub-set of the latent viral genes showed that LMP-1 - the main anti-apoptotic latent protein encoded by EBV - is not involved in the protection afforded here by viral infection. This was confirmed by clones of BL41 stably expressing LMP-1 from a transfected plasmid which respond like the parental cell line. Although steady state levels of Bcl-2 and related proteins varied between BL41 lines and clones, they did not change significantly during apoptosis. Neither was the level of any of these anti- or pro-apoptotic proteins predictive of the outcome of treatment.

We have demonstrated that a subset of EB virus latent gene products (which do not include LMP-1) can inactivate a cell cycle checkpoint for monitoring the fidelity and timing of cell division and therefore genomic integrity. This is likely to be important in EBV-associated growth transformation of B cells and perhaps tumorigenesis.

1515 Effects of human cytomegalovirus immediate early protein accumulation on cell death in the differing contexts of virus infection and cell lines
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In transient assays, the cis repression signal (crs) mediates auto-repression of the human cytomegalovirus (HCMV) major immediate early promoter (MIEP) by the immediate-early 2 (IE2p86) gene regulator. The crs functions during infection, as fibroblasts infected by crs mutant viruses over-accumulated the protein products of the MIEP, IE1p72 and IE2p86. Over-accumulation of IE proteins correlated with the premature apoptotic death of cells late in infection, perhaps reflecting toxicity of over-abundant IE proteins.

Our failure to date to generate stable human fibroblast lines expressing IE2p86 might suggest a similar mechanism of IE2p86 toxicity in this context. Fibroblasts were generated which express a hormone-inducible IE2p86-oestrogen receptor (IE2-ER) fusion protein. Although induced IE2-ER complemented HCMV growth in the absence of IE2p86, hormone induction failed to provoke apoptosis in uninfected IE2-ER fibroblasts. The fate of induced IE2-ER fibroblasts is being investigated, to determine why long-term accumulation of functional IE2 in cell lines is unsustainable.

1600 Anonymised prevalence study of human t-cell lymphotropic virus type 1 and 2 antibody in antenatal clinic attendees at King's Healthcare NHS trust, South East London

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HTLV 1 is found worldwide with areas of high prevalence in Southern Japan, Central and West Africa and the Caribbean. Infection is associated with adult T-cell leukaemia and tropical spastic paraparesis, with a lifetime risk of disease between 2 to 5%. HTLV infection is transmitted vertically via breastfeeding, sexually and by blood transfusion or needle-sharing. The overall rate of vertical transmission is around 20%.

The aim of the study was to assess the prevalence of HTLV antibody in women attending the antenatal clinic between January 1994 and December 1996. Sera from 7,662 women were tested anonymously for HTLV 1 and 2 antibody using the Murex ELISA (GE80/81). Initially reactive sera were confirmed using the Fujirebo Passive Particle Agglutination Test and Innogenetics HTLV _ Inno-Lia, which allowed differentiation between types.

HTLV antibody was detected in 34 (0.44%) sera ; HTLV 1 antibody accounted for 32, with 19 from black Caribbean patients (8 UK born), 10 from black African (2 UK born) and 3 from caucasian patients (2 UK born, 1 born in Jamaica, all 3 with black Caribbean partners); one black African lady was HTLV 2 antibody positive.

These results suggest that HTLV antibody screening in the local antenatal population is an important issue in order to reduce the risks of vertical transmission.

1615 Abundant Tax protein expression in CD4+ T cells naturally infected with HTLV-I is prevented by specific CTL
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Most HTLV-I-infected individuals mount a persistently activated cytotoxic T lymphocyte (CTL) response to the virus. However, it remains unclear whether this CTL response is protective or causes tissue damage. In addition, several observations suggest that HTLV-I is transcriptionally silent in most infected cells, and therefore not detectable by virus-specific CTLs. We show here that i) up to 80% of naturally-infected CD4+ peripheral blood mononuclear cells are capable of expressing the Tax protein of HTLV-I; ii) autologous CD8+ T cells rapidly kill CD4+ cells naturally infected with HTLV-I and expressing Tax in vitro by a perforin-dependent mechanism; iii) the frequency of Tax(11-19)-specific CD8+ T cells is negatively correlated with the percentage of CD4+ T cells in peripheral blood. We conclude that HTLV-I is transcriptionally highly active, and subject to vigorous CTL surveillance in the peripheral blood.

1630 HTLV-I proliferation and translational control in T-cell clones
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The immunosuppressive drug, rapamycin, inhibits translation of a subset of mRNAs characterised by a 4–15 nucleotide polypyrimidine tract (5' TOP) downstream of the CAP site. A 5' TOP comprises an initial cytosine followed by 4–14 pyrimidines. Rapamycin-dependent control involves FKBP12-rapamycin-associated protein (FRAP). Rapamycin forms a gain-of-function inhibitory complex with the FK506 binding protein (FKBP-12): binding of this molecule to FRAP interferes with phosphorylation events and subsequently mRNA translation.

HTLV-I-infected T-cell clones harbouring a transcriptionally active provirus are sensitive to rapamycin (Höllsberg *et al.*, (1992) *J. Immunol.* 148:3256). The 5' viral LTR possesses a 9-nt 5' TOP. We are establishing an *in vitro* model of this rapamycin sensitivity. Through mutagenesis of the viral LTR in the context of a reporter construct, the degree to which LTR sequences are involved in the rapamycin-dependent translational control of HTLV-I is being determined.

1645 Induction of apoptosis in human lung carcinoma cells by the Semliki Forest virus vector and inhibition of tumour growth in nude mice
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Recombinant Semliki Forest virus suicide particles have previously been shown to efficiently induce apoptosis in infected cells, which is due to expression of the nonstructural region of the virus genome. Here we describe preliminary experiments aimed at utilising this property of the SFV vector to construct a tumour therapy agent. The advantage of this system is that the RNA backbone of the vector induces p53-independent apoptosis, so the multicloning site can be used to express other genes that may be important for tumour therapy. Experiments have also been initiated in other laboratories to target the vector to tumour cells. The Semliki Forest virus vector induced apoptosis in human H358a lung carcinoma cells (p53-deficient), as shown by TUNEL staining and the generation of internucleosomal DNA fragments. The EGFP reporter gene was efficiently expressed in such cells. The growth of H358a spheroids in culture, and tumours in nude mice, was efficiently inhibited by administration of such particles.

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0900 Strategies of the baculoviruses for blocking host cell apoptosis

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The baculoviruses encode novel regulators of programmed cell death that promote virus multiplication by suppressing the host cell's apoptotic response to infection. These apoptotic suppressors function in diverse organisms and include the IAPs (*inhibitors of apoptosis*), P35, and recently identified P49. Whereas the IAPs prevent proteolytic activation of apoptotic caspases, the substrate-inhibitor P35 blocks caspase activity and prevents *in vivo* maturation of these death proteases. The crystal structure of P35 revealed a novel reactive site loop (RSL) which is cleaved by the target caspase during inhibition. Distortion of the RSL caused loss of caspase inhibition, but not cleavage. Preliminary studies indicated that P35 undergoes a conformation change upon cleavage of the RSL which may account for its high affinity, post-cleavage binding to the target caspase. Baculovirus P49 shares ~50% amino acid sequence identity with P35. Sequence analyses predicted that P49 also possesses an RSL. Indeed, P49 is cleaved within its putative RSL and forms a stable complex with the target caspase. However, when synthesized *in vivo*, P49 and P35 inhibit different caspases in the death pathway. Structure/function comparisons of the two baculovirus inhibitors should provide insight into the mechanism of caspase selectivity. These studies should contribute towards the development of caspase intervention therapies in the treatment of apoptosis-associated diseases.

0945 What determines the risk of disease in HTLV-I infection?

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Most HTLV-1 infected individuals remain healthy: approximately 5% develop either a rapidly fatal leukaemia or a disabling chronic inflammatory disease such as HTLV-1-associated myelopathy (HAM/TSP). What factors determine the outcome of this infection? There is now good evidence that the risk of inflammatory diseases is strongly associated with the provirus load of HTLV-1. Therefore, the important question becomes: What determines the equilibrium provirus load in an individual?

The relative lack of sequence variation in HTLV-1 suggests that there is a low rate of virus replication, and therefore that the high provirus load is maintained mainly by proliferation of infected cells. Also, it has not been possible to demonstrate Tax protein expression in freshly isolated lymphocytes. These observations suggest that the virus is predominantly latent. However, there is increasing evidence for constant widespread viral transcription. We shall show recent results that suggest that cytotoxic T lymphocytes kill CD4+ T cells that begin to express the virus before they can complete the replicative cycle: in this way the CTL limit the provirus load and therefore the risk of inflammatory disease. The data are consistent with the occurrence of massive CTL-induced death of infected CD4+ lymphocytes *in vivo*. Variation between people in the efficiency of this immune surveillance probably contributes to the variation in disease susceptibility in HTLV-1 infection.

1100 Alphavirus apoptosis: implications for viral pathogenesis

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Cells have evolved pathways to detect infection and linked these to cell death pathways. In a multicellular organism, rapid suicide upon infection can be seen as an altruistic response and a highly effective strategy for limiting infection. However, this strategy would only be effective for non-vital replaceable cell populations. On theoretical grounds alone it might be anticipated that cells such as mature neurons would not trigger a suicide response upon infection. What then are the consequences of virus infection in a mature neurone; viral persistence, neuronal dysfunction and disease?

Continuous cell lines in culture, both insect and mammalian cells, infected with alphaviruses including Semliki Forest virus (SFV) and Sindbis virus (SV) die of apoptosis. The process and the pathways involved have been investigated in detail. Infection of primary cultures of mouse embryonic sensory neurons also results in apoptotic death but an age-related change in susceptibility is observed and adult neuronal populations are frequently resistant to apoptosis. A similar situation exists in the animal; infection of the developing nervous system results in a widespread panencephalitis with neuronal apoptosis whereas, infection of the mature nervous system results in a restricted non-destructive focal infection which can persist without apparent damage even in a mouse with severe combined immunodeficiency.

1145 Infection with BVDV: from inapparent infection to deadly disease

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The genera *Pestivirus*, *Flavivirus*, and *Hepacivirus* constitute the family *Flaviviridae*; the genus *Pestivirus* comprises four species, namely BVDV-1 (bovine viral diarrhoea virus), BVDV-2, classical swine fever virus (CSFV), and border disease virus (BDV). BVDV represents one of the most important pathogens of cattle because it causes significant economical losses worldwide. BVDV infection can have different consequences, such as abortion, diarrhoea, hemorrhagic syndrome, mucosal disease (MD), and most frequently, inapparent courses. According to the effects in tissue culture, two biotypes are distinguished, namely cytopathogenic (cp) and noncytopathogenic (noncp) viruses. Transplacental infection with noncp BVDV can result in the birth of persistently infected animals with an acquired immunotolerance to the original BVDV strain. Such persistently infected animals may come down with MD. In addition to the persisting noncp BVDV, a cp BVDV can always be isolated from animals with MD.

The common thread for all cp BVDV strains is the expression of an 80kDa protein, termed NS3. In noncp BVDV infected cells NS2-3 but not NS3 can be detected. Expression of NS3 therefore is regarded as a marker specific for cp BVDV. NS3 is colinear to the C-terminal part of NS2-3 and is generated by a surprising variety of mechanisms which can often be deduced from the genome structures of the respective cp BVDV strains. The genome alterations identified in cp BVDV genomes include insertions of cellular or viral RNA sequences, deletions of large genomic regions and accumulated point mutations.

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