XI Congreso Internacional de Virología

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La virología ha experimentado avances incuestionables en este siglo, destacándose entre sus logros las vacunas antivirales, que posibilitaron la erradicación de la viruela en 1979 y posibilitarán la erradicación de la poliomielitis en los próximos cinco años. Es por eso que el Prof. D.A. Henderson, que fue asesor del programa para la erradicación de la viruela en el mundo y que anunció la obtención de este extraordinario logro ante la Asamblea General de la OMS, tituló su conferencia en la sesión de apertura del Congreso: "Una carrera llena de obstáculos —El hombre contra los virus". Es evidente que el éxito es esta carrera sólo es posible si se cuenta con el compromiso político de los gobiernos en bienestar de la sociedad. El Prof. Henderson también se refirió al lado oscuro de la virología, en particular al uso del virus de la viruela en la guerra biológica, lo que constituye un reto científico y moral para los virólogos en el desarrollo de medios rápidos y efectivos de prevención, diagnóstico y tratamiento.

El avance y desarrollo tecnológico alcanzado por la biología molecular y la inmunología, junto al desarrollo de un potente instrumental analítico, están imprimiendo una nueva aceleración al desarrollo de la virología, que se evidencia en:

- la manipulación de células de plantas no modificadas, plantas transgénicas y el uso de las plantas como biorreactores para la producción de antígenos y otras proteínas
- la modelación e ingeniería de proteínas
- la caracterización, control y orientación de la respuesta inmune
- los estudios de patogénesis y el desarrollo de modelos animales
- el desarrollo de vectores vivos y virus quiméricos como vacunas
- la inmunización con ADN desnudo.

El Prof. Hilary Koprowski sentenció que el próximo milenio estará marcado por el desarrollo de vacunas en plantas comestibles.

David Baltimore, con destacados logros en el estudio de los retrovirus y quien recibió Premio Nobel por el descubrimiento de la enzima transcriptasa inversa, expuso que se ha evidenciado que el HIV se multiplica en medio de una potente respuesta de linfocitos CD8+, pero que esta respuesta no se mantiene desde el inicio de la infección y es lo que determina que el virus, después de disminuir su multiplicación, la vuelva a intensificar. El HIV reduce la respuesta de linfocitos T citotóxicos mediante la represión de la expresión de las proteínas HLA-A y HLA-B en las células infectadas. La solución de una vacuna contra el sida, estará en lograr mantener la respuesta CD8+ que se genera al inicio de la infección viral. La vacuna debe generar una respuesta de linfocitos T citotóxicos potente y mantenida. Baltimore hizo notar que los estudios de los mecanismos de patogénesis han sustituido al empirismo en la investigación en el campo de las vacunas.

Rolf Zinkernagel, también Premio Nobel, postuló que la respuesta de células T dependía de que los antígenos alcanzaran los órganos linfoides secundarios en una concentración adecuada, y que las células B podían responder, incluso en ausencia de células T, a determinantes antigénicos repetitivos situados a distancias de 1–10nm. Las células B no responden en ausencia de células T a antígenos monoméricos o determinantes no repetitivos. También señaló que podían no existir las supuestas alteraciones de la respuesta inmune que ocasionan la autoinmunidad, sino que la causa está en infecciones virales cuyos antígenos imitan antígenos propios, y señaló que lo que resta es identificar estos virus. Los antígenos propios son generalmente monoméricos y no tienen determinantes repetitivos, y los que los tienen no están accesibles.

Las librerías peptídicas y de fagos están en amplio uso en la obtención de antígenos con mayor inmunogenicidad o mayor afinidad por los anticuerpos, como en el trabajo presentado por C. Howard del Colegio Real de Veterinaria de Londres, donde identificó 29 mimotopos a partir de una librería de fagos que mostraron afinidad por la determinante "a" del antígeno de superficie de la hepatitis B. Este trabajo está encaminado a desarrollar una vacuna peptídica contra la hepatitis B.

Las ventajas del desarrollo de vacunas mediante la construcción de virus quiméricos, se evidenció en los resultados obtenidos por la compañía Oravax en el desarrollo de vacunas contra el dengue y el virus de la encefalitis japonesa, obtenidas mediante la sustitición de los genes codificantes de la proteína de membrana y de la proteína la envoltura viral por sus similares en el genoma de la cepa vacunal del virus de la fiebre amarilla. En ambos casos, se logró protección en primates usando macacos Rhesus como modelo animal.

Stanley Prusiner, quien recibió Premio Nobel por el descubrimiento de los priones, evidenció que las enfermedades neurodegenerativas causadas por los priones son hereditarias e infecciosas. Igualmente, la patogénesis del prión está dada por la conformación que adopta la proteína que lo constituye, lo que también la hace resistente a proteasas. Prusiner postuló que en la similitud epidemiológica, clínica y genética de la enfermedad de Alzheimer y el Parkinson, entre otras, con las enfermedades causadas por priones, se debe encontrar la solución terapéutica para estas enfermedades.

Entre los resultados de los trabajos seleccionados para este número, que evidencian el ritmo que el desarrollo tecnológico antes señalado está imprimiendo a la virología, se encuentran:

- la identificación de receptores celulares para el virus del dengue

Resúmenes seleccionados de las conferencias dadas en XI Congreso Internacional de Virología. Agosto 9–13, 1999, Sidney, Australia.

Selected abstracts of lectures given at 11th International Congress on Virology. August 9–13, 1999, Sydney, Australia.

- combinación de ADN desnudo y poxvirus como vectores vivos en la protección contra el SHIV en primates
- obtención de altos títulos del virus de la hepatitis C mediante el cultivo *in vitro* del virus, partiendo de la hipótesis de que la homología del VHC con los flavivirus debe permitir un tropismo similar en diferentes líneas celulares
- relación entre la respuesta celular y las manifestaciones más serias de la infección causada por el virus del dengue
- caracterización del genoma del virus de la hepatitis A, identificación de los genes y de su papel en la replicación del virus
- obtención de una molécula de interferón β con mayor solubilidad mediante la mutación de ocho aminoácidos para reducir la región hidrofóbica de la molécula.

Es evidente que las nuevas posibilidades tecnológicas y el cúmulo de conocimientos alcanzados, nos permiten adentrarnos como nunca antes en el conocimiento de los virus y de su patogénesis. En los próximos años, debemos esperar importantes resultados prácticos en la prevención, el control y el tratamiento de las enfermedades virales que atacan a los animales, las plantas y el hombre.

Neutralizing antibody-independent containment of immunodeficiency virus challenges by DNA priming and recombinant pox virus booster immunizations

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Eight different protocols for immunization have been compared for the ability to raise protection against immunodeficiency virus challenges in rhesus macaques. The most promising containment of challenge infections was achieved by intradermal DNA priming followed by recombinant fowl pox virus booster immunizations. This containment did not require neutralizing antibody and was active for a series of challenges ending with a highly virulent virus with a primary isolate envelope heterologous to the immunizing strain. Intradermal injections of DNA, but not gene inoculations of DNA, primed protective responses (P = 0.01).

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European hantaviruses: diagnostics and epidemiology

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Only two hantaviruses, Puumala (PUU) and Dobrava (DOB) were seen to cause human disease in Europe according to reliable typing methods (focus-reduction neutralization assays using appropriate hantavirus serotypes or RT-PCR with subsequent sequencing). In addition, Tula virus can infect man but has not been associated with human disease.

We have developed sensitive and specific IgM and IgG EIAs based on baculovirus-expressed PUU, DOB and HTN nucleocapsid (N) proteins. The IgM test is applicable also using peroxidase-conjugated N antigen in an m-capture EIA format and development towards a rapid immuno-chromatography test is in progress. Our data suggest that baculovirus-expressed N protein is antigenically indistinguishable from the native protein and that the whole N protein is more sensitive than a truncated *N*-terminal antigen. Furthermore, although during the first days of the disease IgM, and often IgG antibodies are present, in rare cases (< 2%) PUU IgM antibodies may be negative up to 5 days after onset of illness.

We detected in 1989–1996 7000 PUU infections in Finland (957/year) resulting in an incidence of 19/100,000 (in hyperendemic regions 90/100,000) with < 0.1% mortality. Women contracted the discase at 44 (mean), men at 40 years (male:female ratio 2:1). The PUU antibody prevalence for women entering the maternity clinics nationwide was 3%, but for the whole population endemic areas, 10–20%.

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Rift Valley fever outbreak in Mauritania in 1998

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Rift Valley fever (RVF) is a mosquito-borne viral anthropozoonosis affecting primarily ruminants causing high mortality in young animals and abortions in pregnant females but also humans whose infection leads to a clinical picture ranging from mild febrile case to hemorragic fever. In Mauritania, after a first epidemic in 1987, the virus (RVFV) re-emerged in 1998 from September to December 1998 at Aioun El Atrouss in Hodh El Gharbi Region, where human cases of hemoragic fever and animal abortion, mainly amongst goats, were reported. Epidemiological investigation was undertaken in 4 localities (Chelkha, Vough s, Oum La Hyadh and Oum Kreyia). Eighteen human and 49 animal (sheeps, goat, bovine and camels) cases were diagnosed by IgM ELISA assay and/or virus isolation and/or positive RT-PCR on the S segment. Four and seventeen RVFV strains were isolated respectively for humans and animals cases. Incidence and prevalence of the infection was evaluated respectively by IgM and IgG capture in the 4 localities and in the bordering area in Senegal where IgM positive samples were found, suggesting simultaneous circulation of RVFV in different area of the regions. No RVFV strains were isolated from 1184 mosquitoes and larvae mainly of *Culex* (91.6%) and *Anopheles* genus captured during the last weeks of the outbreak in localities where RVF human and animal cases occurred. RVFV strains from human and animal cases were analyzed by sequencing of the three segments of the genome and exhibit high homology with a strain isolated at Aioun in 1988 suggesting that the virus re-emerged from an endemic focus. Characteristics of the outbreaks in 1987

and 1998 were compared and the potencial mechanism of maintenance of the virus together with the role of molecular tools such as RT-PCR in surveillance for rapid identification of outbreak are discussed.

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Improved bioavailability of a recombinant "Second Generation" interferon-beta

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Interferon-beta (IFN-beta) has a significantly higher antiviral activity than IFN-alpha in some tissues (Heim *et al.*, 1996, J Interferon Cytokine Res 16, 283–287). Moreover, application of IFN-beta may be useful in patients treated with IFN-alpha who developed neutralizing antibodies. However, IFN-beta has to be injected intravenously to achieve significant plasma concentrations and systemic antiviral effects like IFN-alpha. Hence, IFN-beta was not evaluated so far in big clinical trials as an antiviral agent. In order to improve the bioavailability of IFN-beta after subcutaneous injection, a more hydrophilic variant of recombinant IFN-beta was developed. Eight hydrophobic amino acid residues distant to the receptor binding site were exchanged to serine. This recombinant 'second generation' IFN-beta-Var8x was expressed in *E. coli*. Cytopathic effect reduction assays (CPE assays) using encephalomyocarditis virus and A549 cells demonstrated an unchanged specific antiviral activity of IFN-beta-Var8x compared to the parental IFN-beta produced in *E.coli* (IFN-beta-lb). Both IFN-beta-Var8x and IFN-beta-1b were injected in rabbits and plasma concentrations were evaluated by ELISA and CPE assays. There were no significant differences in plasma concentrations of IFN-beta-1b and IFN-beta-Var8x after intravenous injection, but IFN-beta-Var8x plasma concentrations were significantly ($p < 0.01$, Mann-Whitney test) higher than IFN-beta-1b plasma concentrations alter subcutaneous injection of 500 pg. Peak plasma concentrations of 2155 pg/mL (SD 1355) IFN-beta-Var8x were demonstrated by ELISA lh after subcutaneous injection compared to 200 pg/mL (SD 143) in IFN-beta-1b control animals. CPE assays demonstrated 45 IU/mL in plasma lh after subcutaneous injection of IFN-beta-Var8x, whereas < 5 IU/mL were detected with IFN-beta-1b. IFN-beta-Var8x was detectable in plasma for at least 7 h after subcutaneous injection. In conclusion, IFN-beta-Var8x is a promising systemic antiviral agent due to its significantly improved bioavailability after subcutaneous injection and its favourable antiviral activity.

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Biological properties of cytopathogenic hepatitis C (HCV) variants

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Biological properties of Cytopathogenic Hepatitis C (HCV) Variants *in vitro* and *in vivo* models for HCV propagation have been developed. HCV variants isolated from cell cultures chronically infected by RNA HCV-positive blood sera have an ability to induce acute and persistent highly productive HCV infection of cell cultures of different origin as well as mice and rabbits. High pathogenic and haemagglutinating activities were observed with the HCV variants isolated and their capacity to form plaques under an agar overlay was determined. These isolates have been specifically identified as HCV in persistently infected cell cultures and infected mice and rabbits by neutralization tests, enzyme immunoassays, haemagglutination inhibition tests and by RT-PCR and sequence analysis of HCV core regions. Two different types of HCV variants were detected in the culture media of HCV-infected cells: one highly cytopathogenic, the other only slowly pathogenic. HCV neutralizing and HCV haemagglutination inhibition antibodies have been detected in the blood of HCV-infected patients. Infectious DNA was detected during an *in vitro* and *in vivo* persistent infection.

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Role of cytokines in the pathogenesis of dengue hemorrhagic fever

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Dengue virus produces a mild febrile illness, dengue fever (DF), and a severe illness, dengue hemorrhagic fever (DHF). Tbe pathogenesis of DHF is still not clear. This study investigated the role of various cytokines including helper T cell (Th) 1- and Th2-type cytokines, interleukin (IL)-8, IL-12, transforming growth factor-beta1 and the dengue virus-induced cytotoxic factor (hCF) in the pathogenesis of DHF. The levels of different cytokines in sera of the patients were correlated with the duration and the severity of the illness.The sequence of appearance of Th1- and Th2-type cytokines in human peripheral blood leucocyte cultures infected *in vitro* with dengue type 2 virus was also investigated. The data suggest a sequence of events during dengue infection leading to increasing severity of DHF and death of the patients. The most significant finding of the present study was a shift of the predominant Th1-type response observed in patients with DHF to the Th2-type response seen in the patients with DHF grade IV, thus indicating a possible role for Th2 and in the pathogenesis of DHF.

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Receptors for mosquito-borne flaviviruses on human leukocytes

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Diseases caused by arthropod-borne flaviviruses are important emerging infectious diseases worldwide. While the arthropod-borne flaviviruses exhibit considerable homology and very similar replication strategies, they cause distinct disease syndromes in humans, ranging from encephalitis, hepatitis, hemorrhagic syndromes and flu-like symptoms to asymptomatic infections. In flaviviruses cell-attachment is mediated by the envelope (E) protein. Both (glyco-)proteins and glycosaminoglycans (GAGS) have been implicated in dengue and Japanese encephalitis (JE) virus binding, while *in vitro* studies have shown that cells

expressing FcRs can internalize antibody-flavivirus complexes leading to a productive infection. Notably, most of the studies of flavivirus-target cell interaction have been conducted in heterologous systems, and their relevance to human infections is therefore in question. We recently demonstrated differential antibody-independent binding of the four dengue virus serotypes to human leukocytes (Virus Res. 57:63, 1998).

While GAGs might play a role in virus–cell interaction, competition studies showed that not only domain III of the E protein, with a GAG-binding motif, but also epitopes in domain I and II were involved in the virus-binding to human leukocytes, supporting the contention that several host molecules contribute to target cell specificity. Considering the homology of the E protein amongst flaviviruses, we have initiated studies to examine the possibility of shared receptors between the mosquito-borne flaviviruses. Competition studies demonstrated that several viruses of the JE serogroup can interfere with binding of dengue 2 and 3 virus to human macrophages. Further, our results suggest that these viruses may bind to similar surface proteins on human macrophages, however, the affinity and avidity of the binding differ, both between virus types and, at least in some cases, between different isolates of a particular virus.

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Expression, processing and cellular interactions of the hepatitis E virus structural proteins

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Infection by hepatitis E virus (HEV) is the major cause of acute viral hepatitis in the developing world with significant levels of associated morbidity and mortality. The viral genome is a 7.5 kb positive-stranded polyadenylated RNA containing three open reading frames: ORF1 encoding the viral nonstructural polyprotein, ORF2 encoding the major capsid protein, and ORF3 encoding a small protein of unassigned function. In the absence of an *in vitro* culture system for HEV, we have expressed the viral structural proteins in animal cells and characterized their properties. The major structural protein, pORF2, is a ~88 kDa glycoprotein. Endoglycosidase digestion and inhibitor studies suggest that pORF2 is *N*-glycosylated within the endoplasmic reticulum (ER), which also appears to be its major site of accumulation. Site-directed mutagenesis showed Asn-310 to be the major site of glycosylation. pORF2 was found to carry an N-terminal signal sequence, required for its transit through the ER and subsequent glycosylation. The small protein, pORF3, was found to be phosphorylated at a serine residue by MAP kinase, and to associate with the cytoskeleton, suggesting a possible role in the cellular signal transduction pathway. This protein also contains two proline-rich motifs that were found to bind the *src*-homology 3 (SH3) domains found in several intracellular signalling proteins and in cytoskeletal proteins. Results showed *in vitro* binding of pORF3 to the SH3 domains of critical protein tyrosine kinases (PTKS) and cytoskeletal proteins. These studies suggest that pORF3 may behave as an adapter protein, possibly recruiting PTKs to the cytoskeleton. We will present our results on the characterization of pORF2 and pORF3 and their cellular interactions. The results will be analyzed in terms of a model for viral capsid assembly and pathogenesis.

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