Acquired immunodeficiency syndrome

Laying the groundwork for neutralizing AIDS-linked virus

from Jerome E. Groopman

WITH what success does the human immune system produce antibodies that neutralize the human T-lymphotropic virus type III (HTLV-III) — the virus that is aetiologically linked to the acquired immunodeficiency syndrome (AIDS)? And can the neutralizing antibodies be exploited for prophylaxis or therapy of AIDS? Two papers elsewhere in this issue^{1,2} report that such antibodies are present in some sera both from AIDS patients and from people who have been exposed to the virus but are asymptomatic. The relatively low titres of neutralizing antibodies in HTLV-III-infected persons should not dissuade investigators from pursuing their clinical relevance; the feline leukaemia virus often elicits low titres of neutralizing antibodies *in vivo*,³ yet vaccines of some effectiveness, which exploit at least in part this antibody response, have recently been developed.

Several studies that would help to define the significance of HTLV-III neutralizing antibodies *in vivo* immediately suggest themselves. Comparison of the titres and types of these antibodies in asymptomatic and AIDS patients can readily be accomplished. Particularly interesting would be longitudinal studies to examine whether the neutralizing antibodies persist in the sera of those who remain relatively healthy despite exposure to the virus but diminish in those to progress to AIDS. Of course, the clinical situation may not be so simple. Like visna virus, to which it bears some similarity, HTLV-III may alter its surface antigenic determinants over time and 'escape' from the protective effects of neutralizing antibodies^{4,5}. Serial culture of HTLV-III with testing of neutralizing antibodies on the host's own isolates is one approach to determine if *in vivo* there are selection pressures that favour the emergence of antigenically altered virus. Initial reports of apparently healthy adults in Central Africa who are both antibody positive and virus positive for HTLV-III should make this population particularly valuable in addressing the clinical role of neutralizing antibody⁶. Because antigenic determinants on the gp120 envelope protein of the gp41 transmembrane protein of the virus^{7,9} are likely to be the primary sites of interaction with the putative cellular receptor for HTLV-III^{10,11}, it is to be predicted that neutralizing antibodies are directed against gp120 and/or gp41 determinants. The wide antigenic variability in envelope-related antigens from different HTLV-III isolates¹² makes testing the ability of geographically-distant sera to neutralize geo-

graphically-distant isolates a valuable means of identifying conserved epitopes for neutralizing antibodies.

Recently, a retrovirus that cross reacts in serological tests with HTLV-III has been isolated from African Green monkeys¹³. Studies on the neutralization of HTLV-III by sera from these monkeys and of the ability of human HTLV-III neutralizing antibodies to recognize the surface determinants of the monkey virus may also help to localize highly conserved epitopes that would interact with cell-surface receptors for the viruses.

HTLV-III, LAV and ARV — all clearly variants of one type of human retrovirus — have been cloned and sequenced, thereby providing ready access to recombinant (made by recombinant DNA technology) viral peptides^{14,17}. Peptides of the envelope protein can be used *in vitro* to absorb out the neutralizing activity and localize the epitopes that elicit antibodies. It is of interest to determine whether some neutralizing antibodies might react with the putative gene products (termed sor, P', Q and 3'ORF, E', F) that may have functional roles, particularly in the activation of cellular genes (trans-activation). The availability of these products will allow the reverse test: do polyclonal or monoclonal antibodies raised in animals against any of these gene products have *in vitro* neutralizing capacity? To obtain the equivalent human antibodies, it may be possible to use B-cells from people with neutralizing antibodies.

Ultimately, *in vitro* studies will be needed to determine the clinical signifi-

cance of neutralizing antibodies for either prophylaxis or therapy of HTLV-III infection. Initial tests of prophylaxis with hyperimmune globulin and of therapy with pools of high-titre neutralizing antibodies can be undertaken in primates which are susceptible to infection with HTLV-III. Simple vaccination with native or recombinant peptides that elicit neutralizing antibodies may not be sufficient to protect primates against challenge with HTLV-III, but such an approach can readily be examined. The role of the cellular immune response (in addition to the antibody response) in preventing or suppressing human retroviral infection is still unaddressed. Innoculation of primates with forms of HTLV-III that are defective in functional genes but still display structural antigens, or with recombinant constructions of HTLV-III with other viruses, such as vaccinia, should permit the study of both cellular immune and antibody responses.

The HTLV-III research community will now coordinate its clinical and scientific forces to build on the new observation of neutralizing antibodies in the hope of constructing strategies to outwit this threatening human retrovirus. □

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Lasers

Dynamic holograms from crystals

from Malcolm Gower

WHEN certain crystals are exposed to spatially non-uniform light fields, light-induced refractive index changes occur, caused by the migration and re trapping of charges. Known as the photorefractive effect, the phenomenon was first reported nearly twenty years ago as the mechanism responsible for the self-focussing of laser light in crystals of LiNbO₃ and LiTaO₃, and has since been observed for many more crystals, including CdS, InP and GaAs. It was soon recognized that photorefractive crystals used as three dimensional media for holographic recording (volume holograms) could contain extremely high densities of information,

up to 10¹² bits per cm³, yet retain exposure sensitivities comparable to those of the best photographic emulsions (at least 100 μJ per cm²). But, unlike conventional media, photorefractive crystals can be used for dynamic or 'real-time' holography in which the writing and reading processes occur virtually continuously and simultaneously. Recently there have been some remarkable discoveries in the field that may lead to a host of applications in real-time optical data processing — from optical image amplifiers, enhancers and pattern recognizers to optical switches and memories¹.

Unlike the relatively slow serial proces-

sing that occurs in conventional electronic devices, dynamic holograms allow rapid, parallel-bit processing of information. Because the image wave is a complex or phase conjugate of the subject wave, real-time holography is closely analogous to the nonlinear optical method of degenerate four-wave mixing used in producing phase conjugate (wavefront reversing) mirrors (see Fig 1a; ref.2). Thus these crystals can also be used as phase conjugate mirrors to correct distortions on optical wavefronts and to project complex images.

The hologram in photorefractive crystals results from the migrating charges, which may be either positive, as in BaTiO_3 or negative, as in $\text{Bi}_{12}\text{SiO}_{20}$ and $\text{Bi}_{12}\text{GeO}_{20}$. The charges are generated by photoexcitation of electrons or holes from impurity or dopant levels in the band gap of the crystal. The interference pattern set up between the subject and reference waves during recording causes the mobile charges to diffuse or drift (photoconduct) and become retrapped in the darker regions of the interference pattern, leaving behind trapped ionized centres (Fig. 1b). This spatial separation of charge creates local piezo-electrically induced stresses and strains caused by electric fields whose gradients (by Poisson's equation) are a maximum in regions of maximum space-charge density, ρ_{sc} , so that the space-charge field E_{sc} is often phase shifted from the original interference pattern (Fig. 1b). The space-charge field then produces local variations of the refractive index,

Fig. 1 *a*, Dynamic holography: arrangement for producing real-time holograms and degenerate four-wave mixing phase-conjugate mirrors. *b*, Photorefraction: an interference pattern causes charges produced in bright regions to migrate into and become trapped in dark regions, creating a phase-shifted space charge field, which causes variations in refractive index. The case is shown for negative charge carriers. Z is the space coordinate; for other variables see text.

Fig. 2 *a*, Two-beam coupling in phase-shifted holograms. *b*, High-resolution image projector using a self-pumped BaTiO_3 crystal. *c*, Self-pumped, optically bistable phase-conjugate reflector. *d*, Spatial convolution $\odot$ and correlation $\odot$. $\epsilon_1, \epsilon_2, \epsilon_3$ are Fourier transforms of the writing and reading beams E_1, E_2, E_3 .

Δn , throughout the medium as a result of the linear electro-optic Pockels effect. The hologram may be permanently 'fixed', by applying a biasing voltage across the crystal during recording, or can be erased by uniform illumination of the crystal.

The maximum modulation of both the interference pattern and the induced space-charge field occurs when the recording beams have the same intensity, irrespective of their absolute values. Thus, in contrast to most other mechanisms used to produce phase-conjugate mirrors, the normalized image-wave light intensity (phase-conjugate reflectivity) produced by the reading process is insensitive to the absolute intensity of the recording beam. In BaTiO_3 , for example, which has an extremely large electro-optic effect, an image-intensity gain of 100 has been observed using low-powered continuous lasers³. As the crystal behaves like a phase-conjugate mirror with gain, oscillation could be produced between it and a relatively poor, non-aligned reflector, such as a kitchen spatula. When the spatula is moved, oscillation reappears at its new position, demonstrating that the mirror can automatically track its whereabouts.

If the intensity of the recording reference beam is about half that of the subject beam, then during reading maximum reflectivity for transmitted light occurs near the edges of patterns placed in the subject beam, where the intensities of the two recording beams are equal. Such preferential enhancement of the edges of the image is often beneficial when trying to recognize complicated patterns. Because the rates for holographic recording and erasure depend on the photoconductivity of the crystal, which in turn is proportional to the intensity of illumination, using high light intensities for recording and erasure increases these rates. Thus, in BaTiO_3 , for example, a hologram that takes a few seconds to record using a low-powered He-Ne laser requires less than 10 nano-

seconds when an intense (20 MW per cm^2) pulsed laser is used. On the other hand, provided the crystal is kept dark, the recorded hologram can be stored for long periods, from a few hours to over 10 years depending on the crystal species. Storage is limited only by the dark conductivity of the crystal, which eventually redistributes the charge carriers.

If a weak and a strong beam intersect in a crystal (Fig. 2a), most of the energy in the strong beam can be transferred to the transmitted weak beam (since the Bragg reflection of one wave from the hologram is phase shifted by $\pm \pi/2$ from the transmitted wave of the other, the two waves will interfere constructively or destructively if the hologram is additionally shifted by $\pi/2$). Because the volume hologram written in the crystal is often phase shifted from the original optical interference pattern (Fig. 1b), the two writing beams can exchange energy by their self-diffraction from the hologram. This 'two-beam coupling' effect, although complicating the understanding of many observations in photorefractive dynamic holography, leads to some remarkable effects. For example, in BaTiO_3 , where diffusion processes alone cause charge migration, the hologram phase shift is $\pi/2$ and intensity gains of 600 have been observed for the transmitted weak beam. A two-dimensional laser-beam steering device, using two-beam coupling for the potential random accessing of optical memories, has recently been constructed⁴.

Furthermore, two-beam coupling between a single laser beam and scattered light propagating in the appropriate direction in a BaTiO_3 crystal can lead to self-defocussing or 'fanning' of the beam (see cover picture). If there is total internal reflection of the fanned beam from the far corner of the crystal, phase-conjugate reflections can be produced with a reflectivity of up to 30 per cent by using just one input beam to the crystal⁵. We have recently used such a self-pumped phase-conjugate mirror, with the arrangement

shown in Fig. 2b, to project speckle-free images with submicron resolution. Such a system may be useful in photolithography, as used in the production of silicon chips, for projecting high-resolution images over a large area.

Because two-beam coupling effects are inherent in phase-shifted volume holograms, a self-starting, self-pumped phase-conjugate mirror can be produced by feeding back the transmitted fanned beam to the crystal (Fig. 2c; ref. 6). When the intensity of a separate erasure beam is continuously varied, the reflectivity of this device can have multiple values and exhibit hysteresis effects. This optical bistable behaviour may prove useful for high speed switching and memory elements in future optical computers^{7,8}.

By using lenses to focus and take Fourier transforms of the writing and reading beams, dynamic holography has been used to perform the real time operations of convolution and correlation in crystals of $\text{Bi}_{12}\text{SiO}_{20}$ (Fig. 2d; ref. 9). These operations are extremely useful in complex image pattern recognition, and for image deblurring and enhancement.

Furthermore, by using relatively large intensities to illuminate the subject, dynamic holography in photorefractive media can be used to create a beam that

is the mathematical division of two images¹⁰. By making use of the relatively slow recording and erasure times of these media, it is also possible to perform the real-time subtraction and differentiation of images. Compared to the numerical computational techniques now in use, optical images can in principle be manipulated much more rapidly by direct optical methods.

With their many potential applications in dynamic holography, from optical memory storage and switching to image projection and synthesis, photorefractive media may well produce a revolution no less dramatic than that caused by the discovery of photographic materials over a century ago. □

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Immunology

Immune response restored by gene therapy in mice

from Michael Steinmetz

Two classes of cell surface molecules encoded in the major histocompatibility complex (MHC) genes are known to guide T cells of the immune system in their recognition of foreign antigens on cell surfaces. In general, cytotoxic T cells recognize foreign antigens in association with class I molecules, whereas helper T cells co-recognize antigen and class II molecules. The precise molecular interactions that lead to the detection of foreign antigens in the context of MHC molecules (termed MHC restriction) are not known and the role that the MHC antigens play in the development and functions of antigen recognition by T cells is poorly understood. But it is beginning to look as if transgenic mice will provide some answers.

Over the past five years MHC genes, including those encoding for the T-cell restriction elements, have been cloned and sequenced both from mouse and man (see refs 1 and 2). By transfecting genetically distinct cells growing in culture with class I and II genes it has been shown that the molecules they encode function appropriately as T-cell restriction elements³. Currently, these types of gene transfections are being combined with exon-shuffling experiments to identify

which protein domains in class I and class II molecules are recognized by cytotoxic and helper T cells, respectively. One hopes that these experiments, perhaps in conjunction with X-ray crystallography, will eventually define the amino-acid residues that make contact with T-cell receptor molecules and those that may make contact with foreign antigen.

Gene transfer into somatic cells, however, is of limited use. Questions concerning the expression and regulation of genes during development and in differentiated cells, and their influence on the developing individual, are best approached through germline DNA by, in particular, micro-injecting genes into the pronuclei of fertilized eggs (to produce transgenic animals) or using retroviral vectors to transmit genes into early mammalian embryos (or bone marrow cells)⁴. With respect to the immune system there are a series of questions concerning the generation of the diverse repertoire of B-cell and T-cell antigen receptors that can be studied in transgenic animals. In the past two years, several investigators have introduced functional genes for antibody molecules into the mouse germ line to study the tissue and cell specificity of

regulation, and the mechanism of allelic exclusion, of immunoglobulin genes in B cells^{5,7}. Now, transgenic mice have been produced with cloned MHC genes to find out whether the introduced gene can be expressed in a functional way.

Frels *et al.*⁸ have introduced a porcine MHC class I gene into mouse germline DNA and have shown that the foreign gene is accurately expressed in the transgenic mouse. All cells examined display the porcine class I molecule on the surface, and skin transplanted from the transgenic mouse to a normal mouse of the same strain is rapidly rejected. It is known that genetic differences in class I molecules will induce rapid graft rejections — hence they are also termed transplantation antigens. The findings of Frels *et al.* indicate that the porcine class I molecule is recognized by mouse T cells as a transplantation antigen and that the immune system of the transgenic mouse has become tolerant to the porcine molecule. Whether the porcine class I molecule can also function as a restriction element in T-cell-mediated antigen recognition in the transgenic mouse still remains to be investigated.

A related question, namely whether class II antigens encoded by genes introduced into transgenic mice can function as T-cell restriction elements, has now been answered in elegant experiments carried out by three independent groups, two of which describe their results in this issue^{9,10} whereas the third has a paper submitted (L. A. Pinkert *et al.* personal communication). Normally, mice have two different class II molecules, A and E, both of which are composed of distant α - and β -chains. For their experiments, all three groups have made use of strains of mouse that have a 5'-deleted E_α gene and can no longer synthesize the E_α molecule. As a result, their E_β molecules, which are produced as normal, remain in the cytoplasm of the cell rather than forming a heterodimer with E_α on the cell surface. The E_α -deficiency of these mice has previously been correlated with their inability to generate an immune response against certain antigens, for instance to the synthetic copolymer composed of glutamic acid, lysine and phenylalanine (GLPhe).

To perform gene replacement therapy the different investigators used previously cloned intact E_α genes derived from two different mouse strains which contain almost identical E_α polypeptide chains. The intact E_α genes were micro-injected into fertilized eggs of the immune-deficient mice, leading to the birth of a few transgenic mice that contained several copies of the introduced E_α gene. These genes are inherited in a mendelian fashion by the offspring of the transgenic mice, indicating that they are integrated into one autosomal chromosome, and that they are transcribed into messenger RNA molecules of the right size and, more importantly, only in those cells that normally