This Week in SCIENCE

Instrumentation

FOR the past 40 years, *Science* has devoted one October issue to new developments in analytical instruments. The editorial and four articles this week describe some of this year's technologic advances that are making chemical and structural studies of cells, large biomolecules, and small molecular species more detailed and accurate (pages 9, 51, 57, 64, and 71).

Nanolithography

THE sharp tip of the scanner of a scanning tunneling microscope, an instrument that is being used increasingly for explorations of surface topography at atomic scale, has been used for drawing lines and punching holes in the surface of a crystal (page 99). If lasting patterns can be drawn accurately on such surfaces, this technique may facilitate the creation of tiny (nanometer-sized) electronic devices. Garfunkel *et al*. speculate that the modifications to the surface of the conducting oxide—a single crystal of the "blue bronze" $Rb_{0.3}MoO_3$—are brought about by atomic scale abrasions. The early results suggest that the patterns on this oxide are stabler than those that have been etched into other materials such as metals and insulators.

Linked volcanic events

HOT plumes of material rising from deep within the earth's mantle are thought to have a shape that consists of a large spherical head and a long narrow tail. When the head reaches the earth's lithosphere, a large mass of magma can quickly form. Eruption of this magma may result in an accumulation of basaltic lavas, called a flood basalt; continuing magmatism associated with the tail of the plume may maintain a "hot spot" from which magma erupts more slowly, perhaps for hundreds of millions of years. (As crustal plate moves over the tail, chains of volcanic islands, like Hawaii, are generated.) This is the model proposed by Richards *et al*. to account for the association of some of the largest of the continental flood basalts with known hot spots (page 103). Model predictions of plume migration through the mantle correlate with volumes and eruption rates of large flood basalts (proxies for the size of the plume head) and related hot spots. Large volcanic plateaus under the oceans may be the marine equivalents of the continental flood basalts, which also could have formed when the hot mantle plumes first erupted.

Foiling fungi

THE fungus *Lagenidium callinectes* is a formidable pathogen for many different crustaceans. Although its victims include the juvenile and larval forms of the shrimp *Palaemon macrodactylus*, the embryos of such shrimp are remarkably resistant to fungal infections. Symbiotic bacteria (*Alteromonas* species), which live at the surfaces of the externally brooding embryos, provide protection against fungi by secreting an antifungal substance called isatin (page 116). Gil-Turnes *et al*. show that when bacteria and isatin are stripped away, the embryos die of fungus infections; the embryos can, however, be protected if they are exposed to isatin solutions or reexposed to isatin-producing bacteria. The importance of isatin is at least twofold: not only does it preserve the host (a benefit for host and bacteria alike) but it also limits competition at the surface from other pathogens. Surface-associated bacteria such as these may provide chemical protection from dangerous pathogens in the water for a range of aquatic plants and animals.

Defining specificity

AMONG the cells of the immune system that react with viruses are cytotoxic T cells (CTLs). When CTLs encounter AIDS viruses, they react against the virus if receptors on CTLs recognize distinctive peptides of the viral envelope presented in an appropriate context. Takahashi *et al*. have identified a single amino acid in peptides of the AIDS virus envelope protein that can determine which CTLs will react with which peptide (page 118). Two similar peptides, with sequences corresponding to residues 315 to 329 of envelope proteins, were synthesized; each had 15 amino acids, 9 of which were identical. Each peptide showed exclusive reactivity with one population of CTLs. When the amino acid at position 325—which was a valine in one peptide and a tyrosine in the other—was shifted from valine to tyrosine or vice versa, the peptides no longer reacted with their specific CTLs but reacted with those of the other peptide. Thus, although this was not the only difference between the two peptides, amino acid 325 was central to the peptide's specificity. If, through alteration of a single amino acid, a virus can, in vivo, shift its immune specificity, an explanation may be at hand for how some viruses continue for long periods to evade host immune defenses.

Simulation software

TWO software packages for simulations are available for Macintosh computers; their features, differences, strengths, and shortcomings are described by Bogen (page 138). One package, called STELLA, is most useful for processes that have steps that can be compartmentalized; the other, Extend, uses block diagrams connected with input and output arrows to conceptualize systems. These packages can be used for depicting such processes as how sexually transmitted diseases spread from infected to uninfected individuals in a population, how drugs enter the body, move through the gastrointestinal tract, the blood, and the kidneys, and later are excreted, and how biological oscillators may interact to control respiration, circadian rhythms, or the beating of the heart. Through the use of diagrams rather than hard-to-follow and hard-to-alter equations, both packages greatly simplify the procedures for developing, varying, and running models of the dynamical aspects of complex systems.

■ RUTH LEVY GUYER

REPORTS

A single amino acid interchange yields reciprocal CTL specificities for HIV-1 gp160

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Abstract

For the IIIB isolate of human immunodeficiency virus type-1 (HIV-1), the immunodominant determinant of the envelope protein gp160 for cytotoxic T lymphocytes (CTLs) of H-2d mice is in a region of high sequence variability among HIV-1 isolates. The general requirements for CTL recognition of peptide antigens and the relation of recognition requirements to the natural variation in sequence of the HIV were investigated. For this purpose, a CTL line specific for the homologous segment of the envelope from the MN isolate of HIV-1 and restricted by the same class I major histocompatibility (MHC) molecule (Dd) as the IIIB-specific CTLs was raised from mice immunized with MN-env-recombinant vaccinia virus. The IIIB-specific and MN-specific CTLs were completely non-cross-reactive. Reciprocal exchange of a single amino acid between the two peptide sequences, which differed in 6 of 15 residues, led to a complete reversal of the specificity of the peptides in sensitizing targets, such that the IIIB-specific CTLs lysed targets exposed to the singly substituted MN peptide and vice versa. These data indicate the importance of single residues in defining peptide epitopic specificity and have implications for both the effect of immune pressure on selection of viral mutants and the design of effective vaccines.

Comment:

キラーT細胞が認識するHIV-1-IIIB型のエピトープであるP18 IIIB (318-329：RIQRGPGRAFVTIGK) 内の特異性を決定することが判明した325番目のアミノ酸を、患者さんの中で蔓延しているHIV-1-MN株のエピトープP18MN (318-329：RIHIGPGRAFYTTKN)325番目のアミノ酸である芳香族アミノ酸であるY (チロシン) に変換すると、たった一つのアミノ酸の置換によりCTLの特異性が逆転することを報告した。このことは、ウイルスの特異性が、たった一つのアミノ酸により影響をうけること、すなわち一つの変異によりウイルスが免疫システムからエスケープ可能であることを初めて証明したものである。このことは、Science誌の「This Week in Science」でも取り上げられた。

This Week in SCIENCE

Josephson computers

Superconducting Josephson junctions may one day form the basis for ultrafast computers. Hasuo (p. 301) reviews progress that has been made through the use of high-quality niobium junctions. Large-scale integrated circuits have been made that are much faster than existing semiconductor circuits. The first commercial products may be specialized processors and hybrids with existing computers.

Growth hormone and receptor complex

Binding of human growth hormone (hGH) to its receptor stimulates the growth and metabolism of muscle, bone, and cartilage cells; de Vos *et al.* (p. 306; cover) report the x-ray structure of hGH complexed with the extracellular domain of its receptor (hGHbp). Two receptor molecules form the complex with hGH. Although the two hGHbp molecules donate similar residues, the structures of the two binding sites are quite different. The two hGHbp molecules also form extensive carboxyl-terminal contacts, which suggests a sequential mechanism for dimerization that may have implications for signal transduction.

One charge at a time

The ultimate in digital electronics is to use a single electron to change the state of a device; Su *et al.* (p. 313) report the incremental charging of the potential well of a resonant tunneling device by single electrons. In order to separate the effects of size quantization and charging, the submicrometer-sized heterostructure was grown so that one of the barriers of the quantum well was more transparent than the other. When the voltage polarity is chosen so that the emitter was more transparent than the collector, electrons accumulate in the well, and sharp steps are observed in the tunneling current because of Coulomb blockade.

Alkane activation

Measurement of the gas-phase reaction rates for the addition of the normally "unreactive" alkanes to a rhodium complex has verified that these reactions proceed through the formation of a 16-electron intermediate. Wasserman *et al.* (p. 315) irradiated $(\eta^5\text{-}C_5H_5)Rh(CO)_2$ to dissociate one of the CO ligands. Almost every collision of the resulting "naked" complex with alkanes such as neopentane caused activation of carbon–hydrogen (C–H) bonds. In a second step these activated intermediates undergo oxidative addition, breaking the C–H bond.

Cyclic 3' ends

In the major mature form of U6 RNA, one of the small nuclear RNA molecules involved in pre-mRNA splicing, the 3' end has an unusual cyclic 2',3'-phosphate group on a uridyl residue. Lund and Dahlberg (p. 327) identified this apparently post-translational modification through ribonuclease T1 fingerprinting and chemical modification analyses. The formation of the cyclic phosphate may act to inhibit shortening or elongation at the 3' end, thus fixing its length, or may form a transient covalent linkage with another splicesome component.

Coral siblings

Wide distribution, longevity, and clear annual bands of the reef-building coral *Montastrea annularis* has made it the "lab rat" for many ecological, physiological, and geological studies, especially for the poorly understood episodes of coral bleaching. Knowlton *et al.* (p. 330) show that *M. annularis* is really a complex of genetically and morphologically distinct sibling species that differ in important attributes such as coloration and growth rates, a finding that brings into question the widely held assumption that its colony morphology reflects environmental plasticity.

Recognizing more HIVs

Immunization of mice with a recombinant vaccinia virus from one HIV isolate, followed by restimulation of cytotoxic T cells (CTLs) from another isolate with a single substitution at a critical V3 loop residue, generated CTLs with broad specificities. Takahashi *et al.* (p. 333) found that using peptides with aliphatic substitutions at residue 325 during restimulation generated CTLs that could respond to variant sequences at this critical site.

Endosperm evolution

Evidence for the origin of endosperm in flowering plants has been obtained by Friedman (p. 336), who studied the stages of development of the desert shrub *Ephedra* from pollination through formation of cellular protoembryos. Friedman had showed that double fertilization took place in this plant; by serially sectioning 400 ovules, he now shows that the development pattern in *Ephedra* is similar to that in primitive flowering plants, except that the second fertilization product forms additional embryos. This embryo-producing tissue may have evolved into endosperm.

Artificial photoreceptor

Bacteriorhodopsin (bR) films have been used to create image-sensing devices. Miyasaka *et al.* (p. 342; see news story by Flam, p. 289) used Langmuir-Blodgett techniques to coat tin oxide electrodes with bR; the film was covered with an aqueous gel electrolyte and sealed with a gold counterelectrode. These photocells were then constructed into a pixel array to make an image sensor. Like biological photoreceptors, the photocells produce a rectified photocurrent. A constant light intensity produces a rapidly rising photocurrent that peaks and rapidly returns to background. Such response would be useful in optical imaging applications.

17 JANUARY 1992

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REPORTS

Induction of broadly cross-reactive cytotoxic T cells recognizing an HIV-1 envelope determinant

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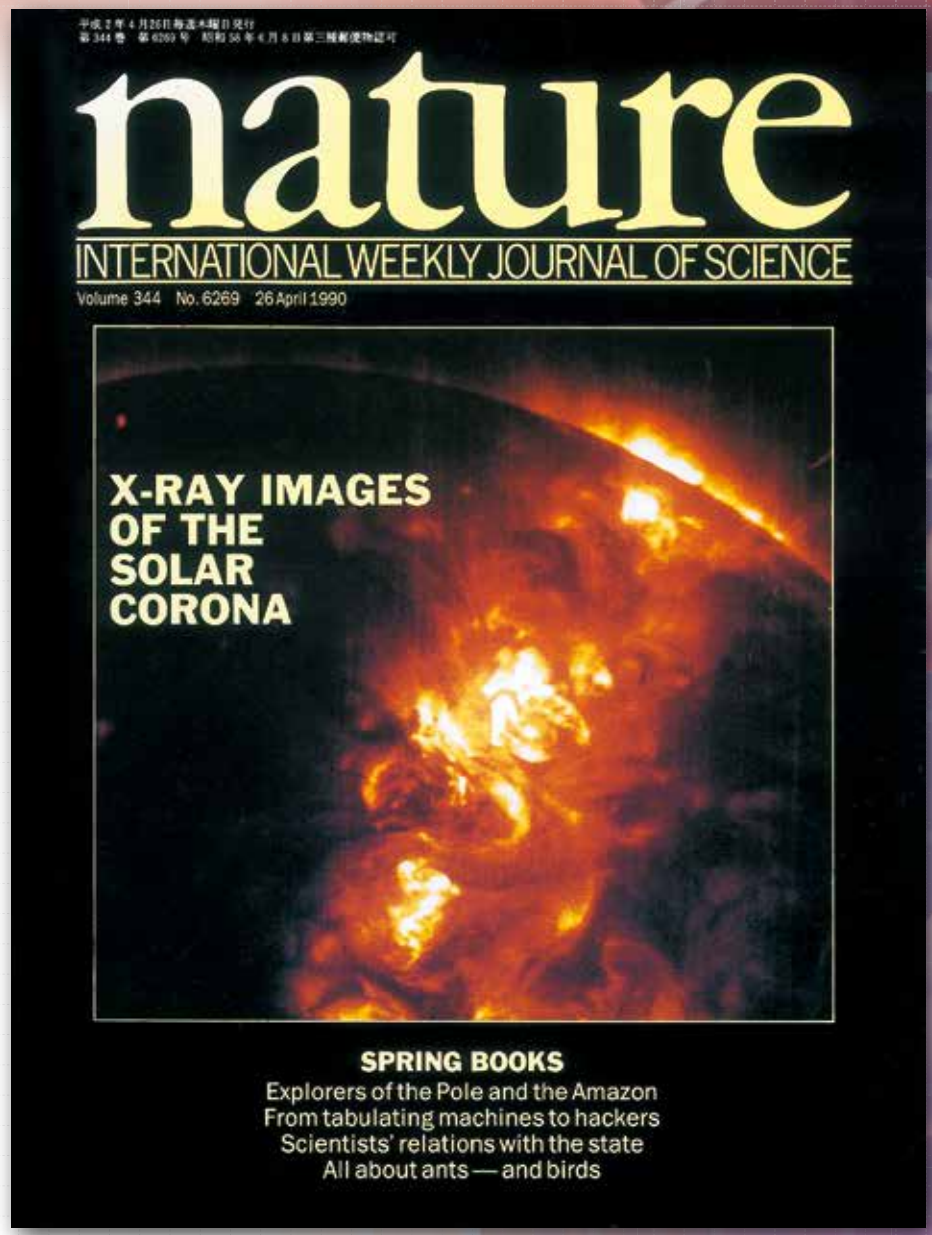
Abstract

An immunodominant determinant for cytotoxic T lymphocytes (CTLs) exists in the hypervariable portion of human immunodeficiency virus-1 (HIV-1) gp160. Three mouse CTL lines (specific for isolates MN, RF, and IIIB) were examined for recognition of homologous determinants from distinct isolates. Only MN-elicited CTLs showed extensive interisolate cross-reactivity. Residue 325 played a critical role in specificity, with MN-elicited CTLs responding to peptides with an aromatic or cyclic residue and IIIB-induced cells recognizing peptides with an aliphatic residue at this position. CTL populations with broad specificities were generated by restimulation of IIIB-gp160 primed cells with MN-type peptides that have an aliphatic substitution at 325. This represents an approach to synthetic vaccines that can generate broadly cross-reactive CTLs capable of effector function against a wide range of HIV isolates.

Comment:

キラーT細胞が認識するHIV-1-IIIB型のエピトープであるP18 IIIB (318-329) 内の特異性を決定することが判明した325番目のアミノ酸である分子鎖型V(バリン)を、別のウイルス株であるHIV-1-MN株の325番目のアミノ酸である芳香族アミノ酸であるY(チロシン）に変換すると、特異性が変化することを1989年のScience誌で報告したが、本論文はHIV-1-IIIB型で免疫したマウスを、HIV-1-MN型のエピトープで再刺激すると、IIIB株ならびにMN株双方に応答するようになる、すなわちImmunizationとBoostingの組み合わせにより特異性が拡大することを報告し、このことは、Science誌の「This Week in Science」でも取り上げられた。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X-RAY IMAGES OF THE SOLAR CORONA
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Scientists' relations with the state
All about ants — and birds

Nature. 1990 Apr 26;344(6269):873-5.

Induction of CD8+ cytotoxic T cells by immunization with purified HIV-1 envelope protein in ISCOMs.

Takahashi H[1], Takeshita T, Morein B, Putney S, Germain RN, Berzofsky JA.

Author information

Abstract

To reduce the risks of immunization with killed or live attenuated virus vaccines, it may be advantageous to use a pure, defined antigen that contains determinants for both humoral and cellular immunity. However, although most non-living intact protein preparations induce antibodies and CD4+ major histocompatibility complex (MHC) class II-restricted helper and/or cytotoxic T lymphocytes (CTL), they do not elicit CD8+ MHC class I restricted CTL. Indeed, with a few exceptions, it has not so far been possible to induce CD8+ CTL by immunizing with intact soluble proteins. We show here that a single subcutaneous immunization in mice with immunostimulating complexes containing either purified intact gp160 envelope glycoprotein of the human immunodeficiency virus (HIV)-1 or influenza haemagglutinin results in reproducible and long-lasting priming of HIV specific or influenza-specific CD8+, MHC class I restricted CTL.

Comment:

従来、細胞内で産生された腫瘍抗原やウイルス抗原はクラスⅠMHC 分子とともに細胞表面に提示され、その抗原を提示した細胞を破壊・排除する CD8 陽性のキラーT細胞を活性化するのに対し、細胞外に放出された抗原分子は、マクロファージや樹状細胞などの抗原提示細胞によって捕捉分解され、その表面に発現したクラスⅡ MHC 分子とともに提示され、その抗原特異的な CD4 陽性のヘルパーT細胞を活性化し、B細胞による抗原に対する特異抗体の産生を促し、異物抗原を排除することが知られていた。本論文は、従来のこうした定説を覆したもので、細胞外で産生された抗原分子が、ある特殊なアジュバント（本論文では、樹皮から採取したサポニン系の免疫賦活物質である ISCOMs(Immuno-stimulating Complex)）とともに抗原を接種した場合、抗原分子はクラスⅠMHC 分子とともに細胞表面に提示され、抗原特異的な CD8 陽性のキラーT細胞が誘導されてくることを、世界で初めて証明した論文。これ以降、腫瘍抗原やウイルス抗原を用いて、これらの抗原を発現した細胞を傷害するような、キラーT細胞の研究が展開されていくことになった。その後、こうした抗原を免疫する場合には、ISCOMs の他にも TLR3 を刺激する ds-RNA、BCG、cholera toxin などのような免疫賦活物質とともに免疫した抗原に対してもキラーT細胞が誘発されることが明らかとなり、現在ではこうした現象を Cross-presentation と呼んでいる。このような事象について Nature 誌では、次に示すような NEWS AND VIEWS で取り上げられた。

NEWS AND VIEWS

HIV IMMUNIZATION

Fresh pathways to follow

Dani P. Bolognesi

THE intricate mechanisms used by the immune system to recognize antigens are of great interest not only to immunologists but also to those involved in vaccine development. The trend over the past several years has been to group antigens into two categories according to the response they induce in the immune system (for review, see ref. 1). Non-replicating entities that enter the antigen-presenting cell from outside (exogenously) are processed in the endosomal compartment and presented at the cell surface in association with class II major histocompatibility complex (MHC) molecules. This activates $CD4^+$ helper T cells which, among other things, are required for the production of antibodies as well as $CD4^+$ class II-restricted cytotoxic T lymphocytes (CTL). Alternatively, antigens enter a different processing pathway which is functional for proteins such as viral proteins that are synthesized inside the cell (endogenously). In this case, association is with MHC class I molecules and it is this complex that primes $CD8^+$ CTL. From the standpoint of immunization with anything other than an infectious agent, it has been something of a dilemma to design non-replicating immunogens that could allow processing and presentation by both class I and class II MHC. But several studies have indicated that these pathways are not as clear-cut as was once thought. For example on page 873 of this issue[2], Takahashi and colleagues now report that a unique subunit immunogen which induces neutralizing antibodies against human immunodeficiency virus (HIV) (B. Morein, personal communication) can also prime MHC class I-restricted HIV-specific $CD8^+$ CTL.

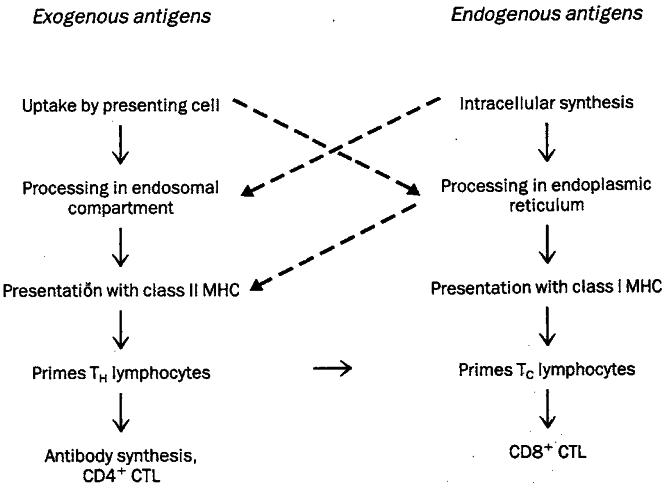
Other examples are to be found in the generation of MHC class I-restricted CTL against soluble ovalbumin[3] and against influenza virus peptides attached to 'lipid feet'[4]. On the other side of the coin is the novel observation that influenza virus proteins processed by the endogenous pathway can associate not only with MHC class I but also with class II molecules[5]. Association of endogenously synthesized antigens with class II MHC also occurs in the case of hepatitis B virus[6] and for HIV envelope antigens synthesized inside the cell[7]. In both of these examples, processing occurs in the endosomal compartment and is therefore distinct from the influenza case[5]. As investigation continues along these lines, models for antigen processing and presentation will probably converge further (see box), as already anticipated[5,7,8].

These studies emphasize the need to understand better the different ways in which exogenous and endogenous antigens are processed and compartmentalized in cells. With regard to exogenous antigens, one might look to some mammalian viruses for clues. Viruses with membrane envelopes can penetrate cells by two mechanisms, one by fusion within endocytic vesicles, the other by direct fusion with the plasma cell membrane. It would be of interest to know how the appropriate entry route is selected. The answer may lie in the viral fusogenic proteins themselves and a more satisfactory definition of their interactions with the cell surface may help us to make cells respond selectively even to inert subunit immunogens. Manipulations with inactivated influenza virus preparations already look promising[9]. Influenza virus normally infects by fusion at low pH in endocytic vesicles, but heat inactivation of the virus causes fusion that allows the viral antigens to bypass the endosomal compartment and so to induce MHC class I-restricted CTL. Curiously, an opposite effect is also possible: for example, live measles virus fuses directly with the plasma membrane and yet preferentially induces a class II-restricted CTL response[10].

The precise mechanisms by which the influenza peptide derivatives[4] and the immune-stimulating complexes (ISCOMs) of Takahashi *et al.*[2] enter cells are not known. Both are associated with lipid, and it could be that the lipid is responsible not only for entry, but also for the susceptibility to processing by the endogenous pathway. On the other hand, as recognition by class II-restricted CTL of endogenously synthesized HIV envelope glycoproteins needs a membrane anchor[8], lipid/protein interactions must be important for delivery of endogenous antigens to the compartments where processing and association with MHC class II products can occur. One could speculate that antigens that remain associated with membranes (both within and outside the cell) may be destined for the class II pathway, whereas those that do not (but can still penetrate cells in the case of exogenous antigens) are channelled to the class I pathway. This might be the case for viral core versus certain envelope antigens, for example. It could also be relevant to measles virus if the dominant CTL epitopes reside in components that associate with membranes.

For HIV vaccine researchers this is welcome news, principally because of the widely held belief that protective immune responses must be directed against both free virus and infected cells. Until now, only attenuated HIV preparations or replicating recombinant vectors were candidates, but the former are excluded because of safety considerations and the latter are still in the developmental stages. The prospect that subunit or peptide immunogens could be endowed with properties enabling them to enter either or both of the main processing pathways and stimulate a full range of immunity will receive considerable attention. One wonders too whether the protective

Pathways of antigen processing and presentation



Solid arrows indicate current models and may represent primary pathways. Alternative pathways (dashed arrows) have recently emerged, the prevalence and efficiency of which may depend on antigen formulation or the nature of the antigen itself. T_H, helper T cell; T_C, cytotoxic T cell; CTL, cytotoxic T lymphocytes.

response obtained with recent inactivated simian and human immunodeficiency viral vaccines[11-13] could have included a CTL component, because the replicating forms of these viruses fuse directly with the plasma cell membrane[14]. But the use of killed HIV preparations for human vaccines carries considerable risk, and structures like the ISCOMs bearing only selected viral components may be attractive alternatives. In this regard the success of using ISCOMs to introduce whole viral proteins into cells for induction of CTL may be important, because it is likely that several T-cell epitopes will be needed both for priming and to overcome allotype restriction. Nevertheless, inclusion of key epitopes may be desirable and the target region described by Takahashi *et al.*[1] is an important one because it is immunodominant for both neutralizing antibodies and CTL. Its drawback lies in its variability, although this might be overcome with appropriate cocktails[15-17].

These results will stimulate a great deal of further research. But their ultimate value may depend on the extent to which the alternative pathways can be used (see figure), particularly in vaccine development and immunotherapy. □

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TRANSCRIPTION INITIATION

In search of the single factor

Marvin R. Paule

EUKARYOTIC RNA polymerases are unable to recognize and transcribe from promoters at the beginning of genes without the aid of additional proteins, the general transcription factors. The three polymerases found in eukaryotic nuclei transcribe different sets of genes, and the number of factors associated with the initiation process increases as the variety of genes transcribed by the polymerase increases. Until now, the sole exception to the requirement for several transcription factors in eurkaryotes was that of ribosomal RNA transcription by polymerase I from *Acanthamoeba*, for which a single ancillary protein (TIF-I) is required[1,2]. But Kassavetis *et al.*[3] now report that only one of the three factors involved in transcription mediated by polymerase III in yeast, TFIIIB, is truly a transcription initiation factor. The others (TFIIIA and TFIIIC) are assembly factors responsible for loading the fundamental factor onto its site on the DNA. This observation means that the mechanisms described for rRNA transcription in *Acanthamoeba*, and now for transcription of yeast polymerase III genes, may be universal for eukaryotic transcription initiation — that is, that only one factor bound upstream of the transcription start site (dubbed + 1) is needed to direct the polymerase to its binding site. The fundamental initiation factor can direct several rounds of initiation, and — most importantly — Kassavetis *et al.* show the additional factors which serve to load it on the promoter are dispensable once assembly of the stable transcription complex has been accomplished.

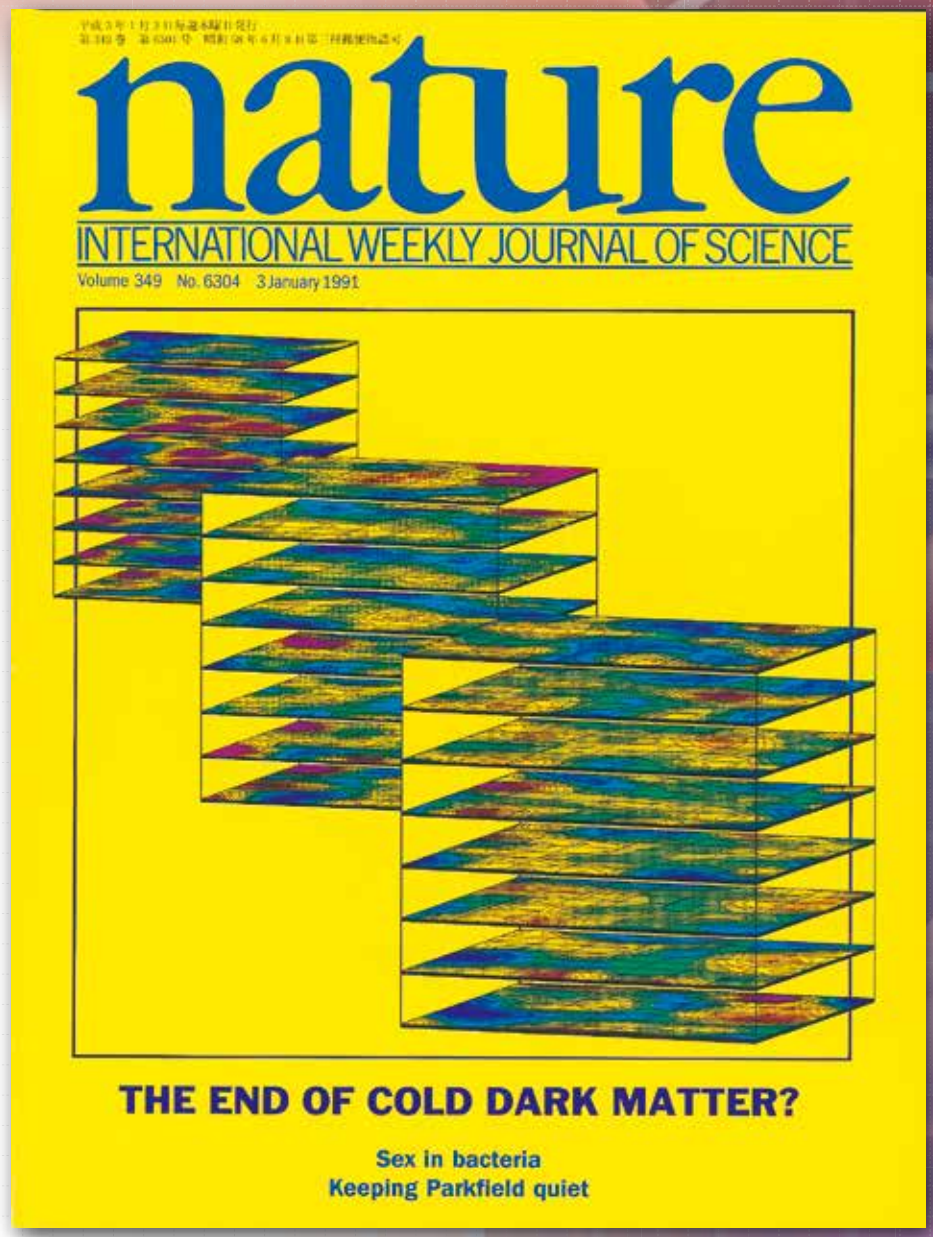
Acanthamoeba TIF-I assembles on the rRNA gene in the absence of additional factors. Yeast TFIIIB, in contrast, cannot load independently onto the template: for 5S RNA genes, TFIIIA and TFIIIC must first bind to DNA, and, for transfer RNA genes, binding of TFIIIC must precede the binding of TFIIIB[4,5]. Similarly, transcription of many vertebrate rRNA genes is stimulated by another factor, UBF, the cloning of which is reported by Jantzen and colleagues on page 830 of this issue. UBF increases binding of the TIF-I homologue; for example, in humans, at least tenfold stimulation of transcription occurs (see Fig.3*d* of Jantzen *et al.*'s report). UBF from *Xenopus* cannot direct transcription alone, but it binds to the core promoter and to the rRNA gene enhancers (60/81 repeats), where it stimulates transcription, presumably by an effect on pre-initiation complex assembly[7-9]. These species differences between the transcription of rRNA in *Acanthamoeba* and vertebrates can be explained by the findings reported for yeast polymerase III.

By taking advantage of the extremely tight binding of yeast TFIIIB once it has assembled on the template, Kassavetis *et al.*[3] show that only TFIIIB is required for transcription by yeast polymerase III. They stripped TFIIIA and TFIIIC from the templates by using high salt concentration or heparin, and then isolated the DNA–protein complexes by size-exclusion chromatography. Only TFIIIB complexes remained, and surprisingly, they retained the capacity for several rounds of transcription. The factors that had been stripped from the template were able to assemble fresh TFIIIB on a new template; TFIIIA and TFIIIC are therefore assembly factors, and are not required for transcription initiation *per se*.

Pre-initiation complexes of tRNA and 5S RNA transcription in yeast[3,5,10] and rRNA transcription in *Acanthamoeba*[2,11,12] have been 'visualized' using footprinting techniques. Yeast TFIIIB was found to protect the DNA template between about 10 and 40 base pairs upstream of +1 on 5S RNA and tRNA genes. The single *Acanthamoeba* rRNA transcription initiation factor (TIF-I) protected between about −12 and −70 base pairs, forming a stable complex which remained bound through several rounds of transcription (see figure). The two factors therefore form similar complexes upstream of the transcription start site.

The first footprints of a eukaryotic polymerase on a promoter demonstrated that *Acanthamoeba* RNA polymerase binds just downstream of TIF-I, protecting over 34 bp, to +18, from DNase I digestion[2]. Replacement of the protected region with a variety of bacterial sequences showed that there are no DNA sequence-dependent contacts made by polymerase, but instead, the enzyme is positioned on the promoter by protein–protein contacts with TIF-I[13]. Similarly, polymerase III protects 23 bp (for 5S RNA genes) or 28 bp (for tRNA genes) of DNA just downstream of TFIIIB. Earlier studies by Sakonju *et al.*[14] suggest that the binding of polymerase to 5S RNA genes is also sequence-independent. To prove that the extended footprints are due to protection by polymerase and not to a conformational change in the previously bound factor, the polymerase I (refs 11, 13) and III (ref. 2) systems were supplied with a nucleotide mixture that allows the polymerase to make only a short RNA product. Addition of a mixture lacking GTP resulted in the polymerase stalling part way down the template. As predicted, the putative polymerase footprints moved part way down the DNA. Addition of all four nucleoside triphosphates resulted in total disappearance of the polymerase footprints.

Significantly, the *Acanthamoeba* TIF-I footprint remains unaltered during initiation, showing that TIF-I remains bound through several rounds of transcription[11,12]. In the polymerase III systems, the TIIIB footprint was also unchanged after partial translocation of the polymerase down the template. Furthermore,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INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
Volume 349 No. 6304 3 January 1991
THE END OF COLD DARK MATTER?
Sex in bacteria
Keeping Parkfield quiet

Nature. 1991 Jan 3;349(6304):74-7.

Excess beta 2 microglobulin promoting functional peptide association with purified soluble class I MHC molecules.

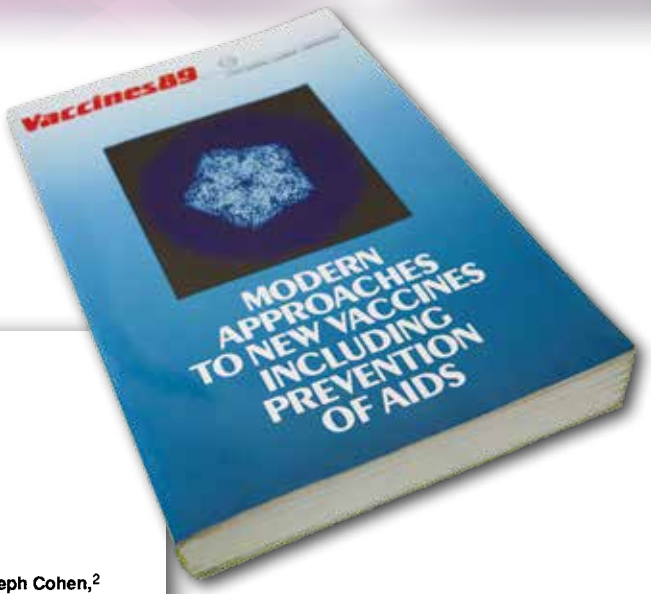
Kozlowski S[1], Takeshita T, Boehncke WH, Takahashi H, Boyd LF, Germain RN, Berzofsky JA, Margulies DH.

Abstract

T lymphocytes expressing alpha beta receptors recognize antigenic peptide fragments bound to major histocompatibility complex class I or class II molecules present on the surface membranes of other cells. Peptide fragments are present in the two available HLA crystal structures and recent data indicate that peptide is required for the stable folding of the class I heavy chain and maintenance of its association with the class I light chain, beta 2-microglobulin (beta 2m), at physiological temperature. To explain how the exogenous peptide used to create targets for cytotoxic cells bearing CD8 antigen could associate with apparently peptide-filled extracellular class I molecules, we hypothesized that stable binding of exogenous peptide to mature class I molecules reflects either the replacement of previously bound peptide during the well documented beta 2m exchange process or the loading of 'empty' class I heavy chains dependent on the availability of excess beta 2m. In either case, free beta 2m should enhance peptide/class I binding. Using either isolated soluble class I molecules or living cells, we show here that free purified beta 2m markedly augments the generation of antigenic complexes capable of T-cell stimulation.

Comment:

クラスⅠMHC 分子の構成成分であるβ2- ミクログロブリン（β2-m）が、抗原を取り込んだ抗原提示分子の構造を安定させる因子であることを初めて見出した報告。このβ2-m は脂質抗原提示分子 CD1 の構成因子でもあり、免疫系を構築する大切な因子と考えられるようになってきた。この後、β2-m の上昇が、免疫応答の重要な指標となることが判明した。



Limited Epitope Repertoire Recognized with Class I MHC Molecules by Murine Cytotoxic T Lymphocytes on the HIV gp160 Envelope Glycoprotein

Hidemi Takahashi,[1] Joseph Cohen,[2] Anne Hosmalin,[1] Kemp B. Cease,[1] Richard A. Houghten,[3] James L. Cornette,[4] Charles DeLisi,[5] Silvia Merli,[2], Ronald N. Germain,[2] Bernard Moss,[2] and Jay A. Berzofsky[1]

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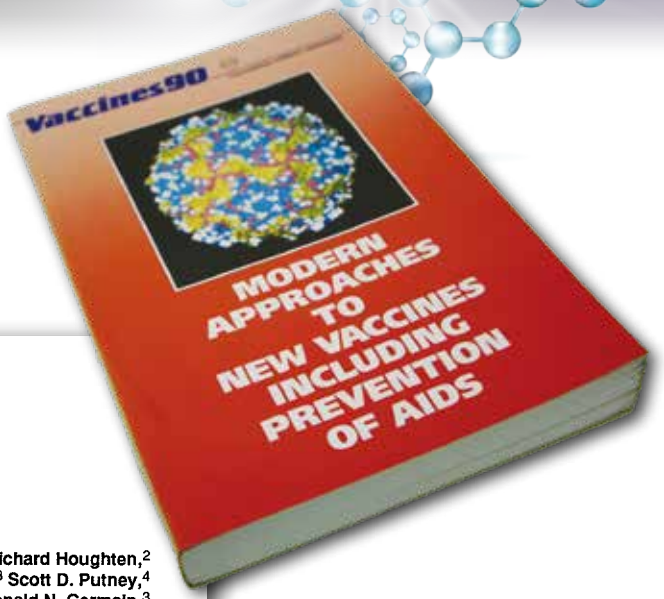
Immunological responses to viral infection include both humoral and cell-mediated effector mechanisms. The major effector cells in cellular immunity are cytotoxic T lymphocytes (CTLs) that kill virus-infected target cells. These cells may be critical for protection against human immunodeficiency virus (HIV) infection because HIV has the ability to be transmitted by cell-to-cell spread without a requirement for free extracellular virus, which would be accessible to antibody (Popovic et al. 1984). However, for most viruses, vaccine design has stressed the elicitation of neutralizing antibodies, although it has been shown that T-cell-mediated immunity, particularly involving class I major histocompatibility complex (MHC) molecule-restricted CTLs, can play an important role in resistance to virus infection (Kast et al. 1986). These features of HIV biology suggest that a vaccine strategy that includes elicitation of specific CTL effectors able to kill cells producing HIV proteins may be particularly useful in preventing successful infection and/or virus spread. Evidence for circulating CTL in HIV-infected individuals has been obtained (Plata et al. 1987; Walker et al. 1987). However, no specific epitopes recognized by CTL in association with class I MHC molecules within any of the HIV proteins had been identified prior to the work described below. Townsend et al. (1986) demonstrated that, like class II MHC-restricted T helper cells, CTLs recognize short

Comment:

Cold Spring Harbor Press から出版された VACCINE 89 に掲載されたのワクチン特集号の 1988 年版。高橋が初めて Cold Spring Harbor で発表した内容が本に掲載された。米国で行った HIV に関する研究が国際的に採用された記念すべき出版物。その内容は PNAS 誌に掲載され、世界中から別刷りの希望が 1,000 件近く届いた。HIV の細胞性免疫の認識部位が HyperVariable な V3 領域に存在することを見出した記念すべき出版物。



Immunodominant CTL Epitope of HIV-1 Envelope Protein: The Relationship between Viral Mutation and CTL Specificity

Hidemi Takahashi,[1] Richard Houghten,[2] Silvia Merli,[3] Scott D. Putney,[4] Bernard Moss,[3] Ronald N. Germain,[3] and Jay A. Berzofsky[1]

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[2]*Torrey Pines Institute for Molecular Studies San Diego, California 92121*

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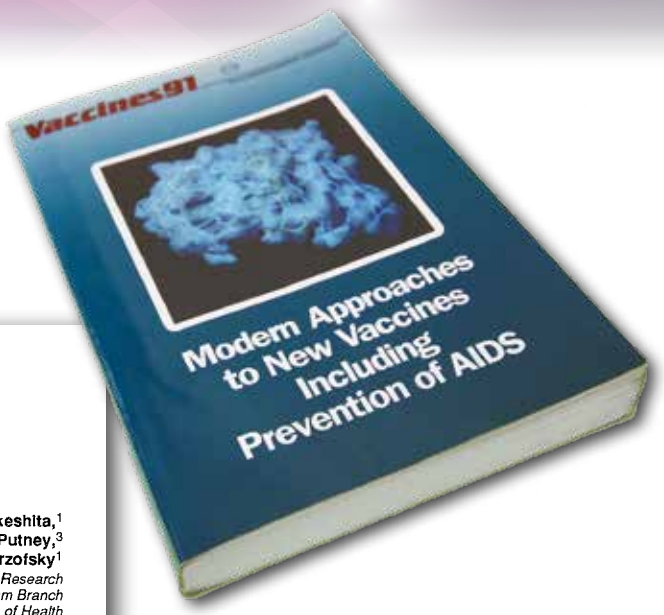
[4]*Repligen Corporation Cambridge, Massachusetts 02139*

Dealing with the problems of both interisolate variability and cell-to-cell transmissibility of human immunodeficiency virus type 1 (HIV-1) constitutes two of the key issues in the design of an effective vaccine for AIDS. Cytotoxic T lymphocytes (CTLs) may be critical for protection against HIV infection because HIV has the ability to be transmitted without a requirement for free extracellular virus, which would be accessible to antibody. Using a series of overlapping peptides covering more than 90% of the gp160 envelope glycoprotein from HIV-1-IIIB isolate, we have found only one immunodominant site for CTLs in mice of the H-2^d haplotype (Takahashi et al. 1988; H. Takahashi et al., unpubl.). This immunodominant determinant is composed of 15 amino acids (18IIIB) (RIQRGPGRAFVTIGK; 315–329 in Ratner numbering [Ratner et al. 1985]) and is restricted by the class I D^d major histocompatibility complex (MHC) molecule (Takahashi et al. 1988). The sequence corresponding to this epitope is highly variable among the different HIV-1 isolates, such as HIV-1-MN (18 MN) (RIHIGPGRAFYTTKN), HIV-1-RF (**TKGPGRVIYATGQ; an asterisk indicates an amino acid deletion), HIV-1-SC (18SC) (SIHIGPGRAFYATGD), and HIV-1-WMJ-2 (18WMJ-2) (SLSIGPGRAFRTREI). For this reason, the homologous sequences of these different isolates need not necessarily be CTL determinants. Nevertheless, we could induce CTLs specific for the HIV-1MN isolate gp160 envelope protein that recognize the corresponding region in HIV-1-MN (18MN) and that are restricted by the same class I MHC molecule (D^d) as IIIB-specific CTLs. Moreover, the anti-MN-specific CTLs did not kill IIIB or RF recombinant vaccinia-virus-infected targets, in agreement with our previous results that a CD4$^-$CD8$^+$ CTL specific

Comment:

Cold Spring Harbor Press から出版されたワクチンの特集号の第２弾 1989 年版（VACCINE 90）に掲載された。HIV 特異的な CTL の認識部位（P18: RIQRGPGRAFVTIGK）の中の赤字で示した 325 番目のアミノ酸 V がウイルス株の特異性を決定し、この部位のアミノ酸が Y に変異しただけで、別の CTL にに特異的なエピトープに変化することを見出した。



A Unique Subunit Immunogen, ISCOM-gp160, Can Elicit MHC Class-I-restricted HIV Envelope-specific CD8+ CTLs

Hidemi Takahashi,[1,5] Toshiyuki Takeshita,[1] Bror Morein,[2] Scott Putney,[3] Ronald N. Germain,[4] and Jay A. Berzofsky[1]

[1]*Molecular Immunogenetics and Vaccine Research Section, Metabolism Branch NCI, National Institutes of Health Bethesda, Maryland 20892*

[2]*Department of Virology National Veterinary Institute Biomedical Center, 5-751 23 Uppsala, Sweden*

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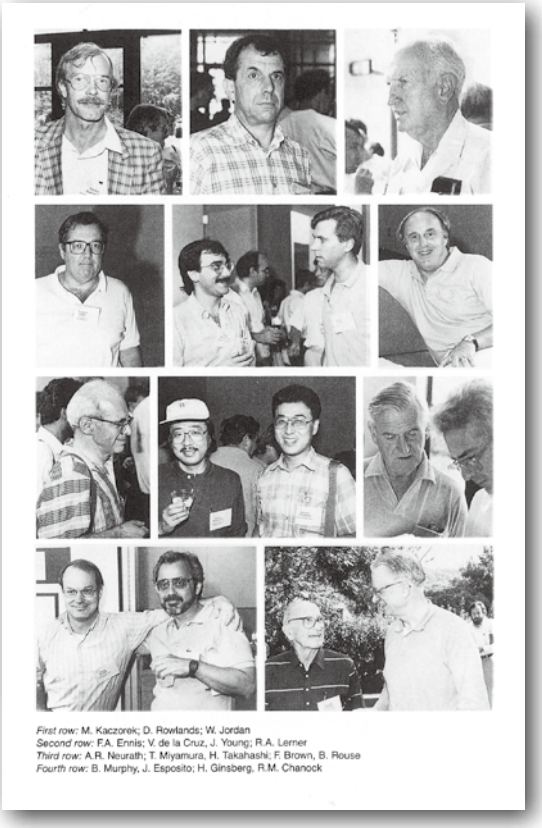
[4]*Laboratory of Immunology NIAID, National Institutes of Health Bethesda, Maryland 20892*

An ideal vaccine should be highly immunogenic and not be harmful to the recipient. However, most current vaccines are composed of killed or live attenuated organisms from which there is a potential risk of infection or induction of autoimmune diseases. To reduce these risks, it may be better to use a pure well-characterized protein that contains immunodominant determinants for both humoral and cellular immunities. In general, to induce these types of immunities, antigens have been divided into two categories (Germain 1986). As shown in Figure 1, most nonreplicating antigen preparations, such as natural or recombinant proteins (myoglobin, albumin, insulin, etc.), that can be taken up by the antigen-presenting cell from outside the cell (exogenously) are proteolytically processed in the endosome to produce fragments that are presented at the cell surface in conjunction with class II major histocompatibility complex (MHC) molecules. This stimulates CD4+ helper T cells, which are required for antibody production, as well as CD4+ class-II-restricted cytotoxic T lymphocytes (CTLs). On the other hand, antigens that are synthesized inside the cell (endogenously), such as viral proteins, enter a different processing pathway and are presented in association with MHC class I molecules to elicit CD8+ class I MHC molecule-restricted CTLs. In the case of human immunodeficiency virus (HIV), which has the ability to be transmitted by cell-to-cell spread without a requirement for free virus, CTLs that eliminate the virus-infected cells may be important for protection. Here, we show that one subcutaneous in vivo immunization with nonreplicating subunit immunogen composed of immunostimulating complexes (ISCOMs) (Morein et al. 1984) containing purified intact gp160 envelope glycoprotein of

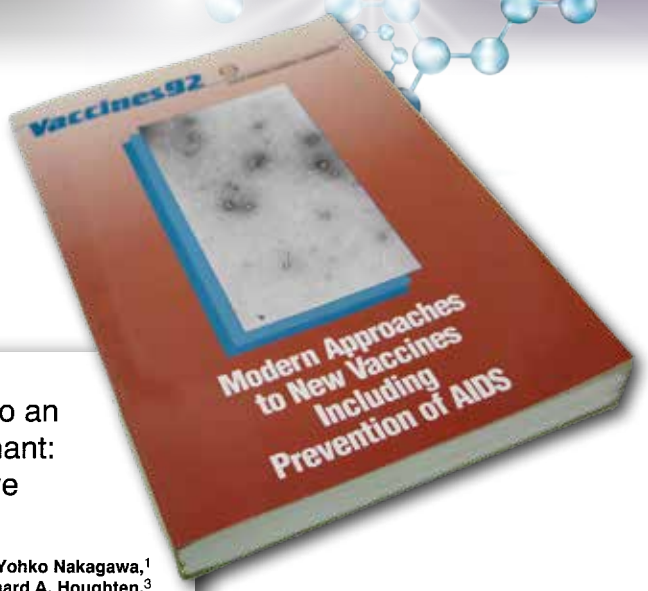
[5]Present address: Department of Microbiology and Immunology, Nippon Medical School, Tokyo 113, Japan.



First row: M. Kaczorek; D. Rowlands; W. Jordan
Second row: F.A. Ennis; V. de la Cruz, J. Young; R.A. Lerner
Third row: A.R. Neurath; T. Miyamura, H. Takahashi; F. Brown, B. Rouse
Fourth row: B. Murphy, J. Esposito; H. Ginsberg, R.M. Chanock

Comment:

これは、Nature 誌にも News & Views で紹介されたことであるが、樹皮からとったサポニン系のアジュバントが、抗体ではなく、特異的キラー T 細胞を誘導することを示した内容で、権威ある Cold Spring Harbor での会議において、トップバッターとして 30 分近い発表の機会を提供されたもの。日本からニューヨークへ行き発表の機会を与えられた。この内容が掲載された Vaccine91 誌に私自身の顔写真も掲載された。一緒に写っているのは後に C 型肝炎ウイルスの発見者として国立感染症研究所の所長を務められた宮村達男先生。



Analysis of CTL Cross-reactivity to an HIV-1 Immunodominant Determinant: Elicitation of Widely Cross-reactive CTLs

Hidemi Takahashi,[1] Yohko Nakagawa,[1] Charles D. Pendleton,[2] Richard A. Houghten,[3] Kozo Yokomuro,[1] Ronald N. Germain,[4] and Jay A. Berzofsky[2]

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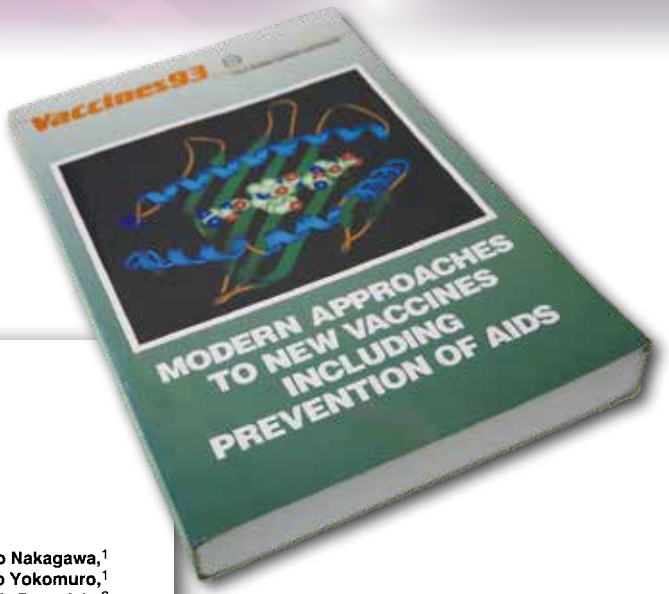
The progress of vaccine development against human immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS, has been impeded by the rapid variation in the sequence of viral antigenic determinants that are seen by immune effector mechanisms, such as neutralizing antibodies and cytotoxic T lymphocytes (CTLs). This variability is especially high in the V3 domain of the surface envelope glycoprotein gp120, to which we have localized an immunodominant epitope for $CD8^+$ CTLs in both humans (Clerich et al. 1991) and mice to residues 315–329 (Takahashi et al. 1988) (Ratner numbering) (Ratner et al. 1985). Interestingly, this site overlaps the target of the major neutralizing antibodies (Goudsmit et al. 1988; Matsushita et al. 1988; Palker et al. 1988; Rusche et al. 1988) and also contains a helper T lymphocyte determinant (Takahashi et al. 1990). Thus, this region seems to be useful as a component of a synthetic vaccine. However, because both neutralizing antibodies and CTLs to this site act only in a type-specific manner, antibodies or CTLs specific for an HIV-1 isolate will usually lose efficacy after selection of viral mutants at these critical sites. Therefore, it is important to investigate means of broadening the specificity of immune effector responses. From this point of view, we have examined the cross-reactivity of raised CTLs to a variety of HIV-1 isolates. In addition, we describe a new method to induce CTLs that react with wide range of variant sequences at the immunodominant CTL determinant.

CTL Cross-reactivity to the Homologous Portions of gp160 from Different HIV-1 Isolates

Among several cloned HIV-1 isolates, we have selected three different variants (IIIB, MN, and RF) and have established three non-cross-reactive CTL lines from BALB/c (H-

Comment:

それまでウイルス特異的 CTL は、交差性を示さず単一特異的なものが主体であったが、CTL の中には交差性を示すものがあり、どのような株に最初に罹患し、次に罹る株によって、双方に反応する CTL が誘導されるかを見出し、その内容は VACCINE 92 に掲載された。その後、どのウイルス株に最初にかかるかということは、その後の免疫応答の方向性を決定する（Original Sin: 抗原原罪）という言葉によって表現されている。



Elicitation of CD8+ Class-I-restricted CTLs by Immunization with Irradiated HIV-1 Peptide-pulsed Dendritic Cells

Hidemi Takahashi,[1] Yohko Nakagawa,[1] Masato Takeuchi,[1] Kozo Yokomuro,[1] and Jay A. Berzofsky[2]

[1]*Department of Microbiology and Immunology Nippon Medical School, Tokyo 113, Japan*

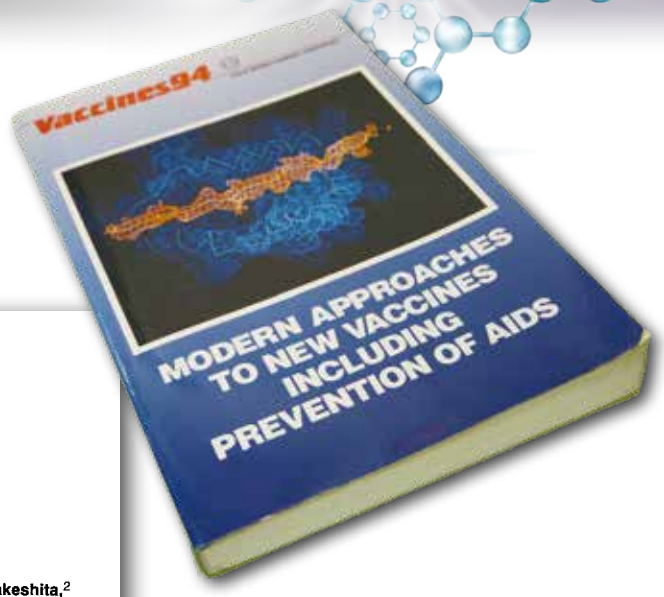
[2]*Molecular Immunogenetics and Vaccine Research Section, Metabolism Branch NCI, National Institutes of Health Bethesda, Maryland 20892*

In general, endogenously synthesized viral antigens are fragmented by antigen-processing enzymes in the cytoplasm or endoplasmic reticulum and are presented on the surfaces of virus-infected cells in conjunction with class I major histocompatibility complex (MHC) molecules. Such processed peptides of viral proteins associated with class I molecules can be recognized by $CD8^+$ cytotoxic T lymphocytes (CTLs) that kill the virus-infected cells. This mechanism allows virus-specific CTLs to contribute to the recovery from and protection against many types of viral infections. To obtain such CTL responses, in vivo priming with replicating infectious agents is usually required. In the case of human immunodeficiency virus (HIV) vaccination, the utilization of such a live virus may be too risky even though attenuated, since such retroviruses have a potential risk of integrating the viral genome into the host cellular chromosomes and may induce immune disorders. Thus, pure, well-characterized antigenic proteins or synthetic peptides that contain immunodominant determinants may be preferable to use for induction of such CTLs. However, $CD8^+$ CTLs are usually thought to be very difficult to generate with nonreplicating subcomponents such as viral proteins or peptides.

We reported that we could elicit such $CD8^+$ CTLs with a single subcutaneous immunization of an immunostimulating complex (ISCOM) (Morein et al. 1984) containing the purified envelope gp160 protein of HIV-1 (Takahashi et al. 1990b). However, the gp160 protein, composed of about 900 amino acids, contains the sites for antibodies that enhance viral replication (Takeda et al. 1988; Robinson et al. 1990) as well as an immunodominant site for CTLs that we identified as a 15-residue peptide (peptide 18IIIB: RIQRGPGRAFVTIGK) in BALB/c ($H\text{-}2^d$) mice (Takahashi et al. 1988) which is also seen by human CTLs (Clerici et al. 1991). Therefore, peptide 18IIIB may be used as a component of a subunit vaccine to elicit virus-specific CTLs. By making use of the fact that CTL precursors do not seem to distinguish between virus-infected cells and virus-derived peptide-pulsed cells, we show here that by immunizing with peptide-pretreated syngeneic cells we could prime $CD8^+$ class I MHC-molecule-restricted CTLs specific for this site without using a harmful adjuvant (H. Takahashi et al., in prep.).

Comment:

特異的キラーＴ細胞が、抗原ペプチドを結合した樹状細胞によって誘導されることを世界で初めて報告した文献。この後、樹状細胞がウイルスのみならず、腫瘍に対する細胞性免疫誘導の鍵を握る細胞として注目を集めることとなった。日本では本研究の内容が、各種の新聞やテレビ・ラジオのニュースとして取り上げられ、注目を集めた。



An Immunodominant Determinant of HIV-1 Envelope Recognized by Both Class-I-restricted CD8+ CTLs and Class-II-restricted CD4+ Helper T Cells Shares Similar MHC-binding Sites

Hidemi Takahashi,[1] Toshiyuki Takeshita,[2] Steven Kozlowski,[3] Yohko Nakagawa,[1] Jeffery Ahlers,[2] Charles D. Pendleton,[2] Richard L. Moore,[4] Barbara S. Fox,[4] Kozo Yokomuro,[1] David H. Margulies,[3] and Jay A. Berzofsky[2]

[1]*Department of Microbiology and Immunology Nippon Medical School Tokyo 113, Japan*

[2]*Molecular Immunogenetics and Vaccine Research Section, NCI, National Institutes of Health Bethesda, Maryland 20892*

[3]*Molecular Biology Section Laboratory of Immunology NIAID, National Institutes of Health Bethesda, Maryland 20892*

[4]*Department of Medicine University of Maryland Medical School Baltimore, Maryland 21201*

Antigen-specific T cells appear to see processed peptide antigen in conjunction with molecules encoded by the major histocompatibility complex (MHC) on the surface of the cells. There are two distinct MHC molecules, class I and class II, and two different antigen-processing pathways are thought to deliver peptides to these MHC molecules (Germain and Margulies 1993). In general, $CD8^+$ cytotoxic T lymphocytes (CTLs) can recognize antigenic peptide presented by class I MHC molecules, whereas $CD4^+$ helper T cells can see such peptide presented by class II MHC molecules. In addition, the length of epitopic peptides presented by class I molecules is usually about eight to ten residues, whereas the length of peptides presented by class II appears to be longer. Therefore, it is generally thought that $CD8^+$ CTLs and $CD4^+$ helper T cells may respond to different determinants within an antigenic protein. One can also assume that a peptide which binds to a class I MHC molecule will not necessarily bind to a class II MHC molecule, and vice versa. Nevertheless, we have found that a peptide corresponding to an immunodominant determinant of human immunodeficiency virus type-1 (HIV-1) (IIIB isolate) envelope gp160 protein (315–329) (18IIIB: RIQRGPGRAFVTIGK) (Takahashi et

First row: R. Chanock; E. Norrby, F. Brown
Second row: A.W. Funkhouser; H. Takahashi, H. Ginsberg, J.A. Berzofsky; E.K. Subbarao
Third row: R. Austrian, J.B. Robbins, P.F. Sparling, J.T. Poolman

Comment:

私が見出したエイズウイルスの特異的認識エピトープは、CD8 陽性 CTL のみならず、CD4 陽性ヘルパー T 細胞の認識部位でもあり、非常に興味深いエピトープであることが判明した。この研究内容は VACCINE 94 に掲載されたが、一緒に写っているのは、米国での指導者であった Jay Berzofsky 博士と国際的な科学者 Ginsberg 先生。