constants of 7–14 and 0.4–1 ms, ($V_m = -90$ to -70 mV), respectively, and the estimated values for the single channel conductance were 2–3 pS.

As shown in Fig. 3, the absence of NMDA channels was confirmed in isolated patches exposed to NMDA (30 μM, four patches) or asparate (30–100 μM, two patches); the same patches invariably gave currents with glutamate, quisqualate or kainate (Fig. 3*b*). Furthermore, the possibility that trypsin treatment of cells[5] caused a selective loss of NMDA receptors[22] was excluded by experiments such as that depicted in Fig. 4*a*, showing that type-2 astrocytes in mechanically dissociated explant cultures[19] were also insensitive to NMDA. Figure 4 illustrates a direct comparison of the channels activated in granule neurons and in type-2 astrocytes, present in the same explant cultures. In the granule cells, NMDA and glutamate produced currents that opened predominantly to levels above 30 pS. Although Mg^{2+} blocks the NMDA-activated 45-pS conductance in neurons[13], in cerebellar type-2 astrocytes Mg^{2+} (0.5 mM) did not induce a flickery block of any of the multiple levels (including the 45-pS levels) activated by quisqualate (eight patches).

Our results demonstrate that type-2 astrocytes have both quisqualate- and kainate-activated channels. In certain respects these are clearly similar to neuronal glutamate channels[11,12] but the data also suggest some differences. For example, certain neurons lacking NMDA receptors fail to give 45-pS openings in response to quisqualate[23]. By contrast, in the astrocytes, 45-pS events were readily obtained with quisqualate, despite the absence of NMDA receptors. Moreover, the fact that Mg^{2+} blocks NMDA 45-pS events in neurons[13] but not the quisqualate 45-pS levels in astrocytes, raises the intriguing possibility that some quisqualate 45-pS openings may be Mg-insensitive in neurons (see also Fig. 4 legend). Kainate currents in type-2 astrocytes differed from those described in some neurons[11,12], in the lack of openings above 30 pS. This may reflect differences in the kainate channels present in the two cell types, or it may indicate that in neurons kainate events above 30 pS are produced by the activation of NMDA[11] or quisqualate receptors. As well as possessing amino-acid receptors, type-2 astrocytes show some other 'neuronal' characteristics both in their antigenic properties[24,25] and in their voltage-activated channels[26]. Our results further suggest that a proportion of glutamate receptors, or messenger (m) RNA encoding these receptors, derived from whole brain tissue may originate from glia. This could be one reason why mRNA injected into *Xenopus* oocytes induces a low density of NMDA receptors[27,28], compared with quisqualate and kainate receptors.

What is the functional significance of the astrocytic glutamate receptors? The type-2 astrocytes possess end-feet, in close apposition to the nodes of Ranvier *in vivo*[9,10], that are well positioned to be activated by any axonal release of transmitter. Such release of glutamate from axons has previously been reported[29,30]. Our finding that the receptors remain active in the continued presence of glutamate suggests that any axonally released transmitter acting on nodal astrocytes would cause a steady change in the local ionic environment, and in electrical excitability of the axon. In addition, the ability of type-2 astrocytes to 'detect' or respond to glutamate may be important in the formation or maintenance of the node; such responses may also play a role in the interactions between axons and migrating progenitors of the type-2 astrocytes, which also seem to possess glutamate receptors[5]. □

Received 20 March; accepted 24 April 1989.

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ACKNOWLEDGEMENTS. We thank Corinne Symonds for help with cell culture and antibody labelling, Alistair Mathie, David Colquhoun, Barbara Fulton and Martin Raff for helpful comments and discussions. This work was supported by the Wellcome Trust, the MRC and EMBO (V.G.).

Interleukin-2 production used to detect antigenic peptide recognition by T-helper lymphocytes from asymptomatic HIV-seropositive individuals

Mario Clerici*, Naomi I. Stocks*, Robert A. Zajac†, R. Neal Boswell†, Denise C. Bernstein*, Dean L. Mann‡, Gene M. Shearer* & Jay A. Berzofsky§

* Experimental Immunology Branch, ‡ Viral Carcinogenesis Branch and § Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda Maryland 20892, USA
† HIV Unit/SGHMMM, Wilford Hall, Lackland AFB, Texas 28236, USA

T LYMPHOCYTES from mice[1] and healthy humans[2] immunized against the human immunodeficiency virus (HIV) envelope have recently been shown to recognize two antigenic regions of the gp160 HIV-envelope protein which have been located on the basis of amphipathicity[3–6]. In HIV-infected humans, T-cell proliferative responses are lost soon after infection[7,8]. Here we demonstrate that interleukin-2 production is often retained even when proliferative activity is absent, and that it can be used to monitor T-helper cell responses by HIV-seropositive donors. We use this approach to investigate the T-helper cell response of 42 asymptomatic HIV-seropositive patients to four synthetic gp160 peptides and to influenza A virus, an antigen requiring intact CD4 T-helper cell function. As many as 67% of the HIV-seropositive donors who retain responsiveness to influenza A virus respond to a single peptide, and 85–90% responded to at least one of the peptides.

We tested the ability of peripheral blood leukocytes (PBL) from asymptomatic, HIV seropositive (HIV^+), Walter Reed Stage 1 and 2 patients (all of whom had >400 $CD4^+$ cells mm^{-3}) (ref. 9) to produce interleukin-2 (IL-2) when stimulated *in vitro* with influenza-A virus (FLU), or with four HIV envelope peptides: T1, T2, TH4.1, and P18 (Fig. 1; Table 1). The PBL from an HIV^- donor (Fig. 1*a*) generated a strong IL-2 response to FLU, but failed to respond to any of the HIV synthetic peptides. PBL from a Walter Reed Stage 1 patient (1,463 $CD4^+$ cells mm^{-3}) (Fig. 1*b*) responded as well to FLU as those from the HIV^- control, and also generated strong IL-2 responses to T1 and TH4.1, with lower but positive responses to T2 and P18.

TABLE 1 T-helper cell responses of PBL from HIV seropositive and seronegative donors to influenza A virus and four HIV synthetic peptides

Donor number	IL-2 production response					Proliferation response				
	FLU	T1	T2	TH4.1	P18	FLU	T1	T2	TH4.1	P18
363*	6.7	9.5	2.8	2.3	3.1	2.7	1.6	2.9	0.3	0.4
471	2.6	2.2	2.8	1.6	3.7	0.4	0.2	0.8	0.2	0.2
136	2.3	2.5	0.6	0.3	1.1	1.1	0.8	0.4	0.4	0.8
300	2.1	0.8	0.8	1.2	1.0	1.0	0.3	0.3	0.2	0.3
346	2.1	1.9	1.8	2.2	2.1	3.5	5.2	2.4	2.2	2.3
429	6.4	2.1	2.1	1.6	1.0	6.4	2.5	1.2	0.8	1.1
149	6.2	9.6	1.1			1.5	1.5	1.1		
146	4.7	2.7	3.0			0.9	0.5	0.6		
318	12.0	0.9	1.0			0.9	0.8	0.7		
320	5.5	1.1	0.8			1.7	0.4	0.5		
326	3.7	3.2	0.8			3.5	0.7	1.4		
382	19.1	20.0	24.1			0.9	0.8	0.9		
331	6.2	1.4	2.2			1.1	0.7	0.8		
108	2.3	2.1	1.5			0.6	2.0	2.3		
186	2.2	1.7	2.7			0.8	1.1	0.8		
250	4.9	5.5	2.8			2.4	1.9	1.5		
425	3.1	2.0	2.3			1.1	2.2	1.8		
253	96.8	2.1	2.7	4.5	7.8					
772	2.8	2.5	2.1	2.9	1.6					
399	2.9	1.4	3.4	2.8	2.4					
LW	4.5	2.8	0.7	2.2	1.2					
513†	1.1	0.7	0.9	1.1	1.2	1.1	0.3	0.8	0.1	0.1
308	0.9	0.6	0.7	1.1	0.6	1.3	0.5	0.5	0.4	0.8
353	1.8	0.9	1.1	0.8	1.1	1.2	1.2	1.1	1.3	0.4
757	1.1	1.4	1.4	1.7	1.2	1.4	0.4	0.6	1.7	1.8
1	1.9	0.9	1.1	1.0	0.6	0.6	0.9	1.4	0.5	1.1
304	1.4	1.0	0.8	1.2	0.9	0.7	1.5	1.0	0.4	1.1
671	1.6	1.0	1.2	2.0	1.7	1.3	0.9	1.3	0.7	1.3
417	1.6	1.5	1.0	1.0	0.7	1.1	1.0	1.2	1.0	1.4
267	1.7	1.6	0.9	0.6	0.5	1.2	1.1	1.3	0.4	0.3
79	1.6	0.8	0.8	0.8	1.4	1.1	0.8	1.5	0.1	0.1
327	1.9	1.3	1.2	1.7	1.6	2.5	1.5	1.4	0.1	0.1
516†	1.8	1.5	1.4	1.2	1.9	1.4	0.6	1.4	0.1	0.1
575	0.9	0.8	0.5	0.9	0.8	0.9	1.7	2.2	0.1	0.1
176	1.3	1.4	1.1	1.3	2.0	2.9	1.2	0.2	0.5	0.2
415	1.9	1.4	0.9	0.7	1.0	1.2	1.3	0.6	0.8	1.0
756	1.0	0.8	0.8	0.8	0.9	0.7	1.3	1.2	0.2	0.3
328	1.8	1.0	1.6			0.9	1.4	1.0		
20	1.2	1.6	0.6			0.8	1.2	1.2		
769	1.6	0.6	1.0	0.9	1.0					
352	1.0	0.4	0.5	0.8	0.9					
373	1.7	0.8	0.3	1.0	0.9					
43‡	9.8	0.4	0.3	0.8	0.3	23.7	0.5	0.3	0.2	0.2
44	12.1	0.8	0.9	0.1	0.2	6.8	0.5	0.6	0.7	0.7
45	10.9	1.3	1.2	0.9	0.8	8.7	0.9	1.3	0.6	1.0
46	5.7	1.0	0.9	1.1	1.0	4.1	0.8	0.4	0.4	0.8
47	5.7	0.7	0.8	1.0	1.1	55.9	0.5	0.5	0.4	0.2
48	4.6	1.6	0.3	0.3	0.2	42.3	0.8	0.6	0.2	0.1
49	3.4	1.1	1.1	0.7	0.6	3.7	0.1	0.2	0.3	0.2
50	3.1	1.1	0.2	0.5	0.7	15.2	1.1	0.9	0.8	1.0
51	2.9	0.4	0.5	1.0	0.8	19.9	0.7	0.6	0.8	0.2
52	4.3	0.4	0.3	0.2	0.2	7.7	0.8	0.5	0.9	0.5
53	4.4	0.8	1.1	0.2	0.3	17.1	1.1	1.0	0.4	0.3
54	7.8	0.1	0.1	1.1	0.3	5.9	0.1	0.1	0.1	0.2
55	7.9	0.3	0.2	0.6	0.2	5.9	0.1	0.1	0.1	0.2
56	6.4	1.0	1.2	0.8	0.6	6.8	0.6	0.7	0.8	0.6
57	5.8	1.0	0.7	1.3	0.4	7.3	0.4	0.3	0.3	0.2
58	10.5	1.0	1.2	0.3	0.3	7.6	0.4	0.4		
59	5.6	1.2	1.0	1.1	0.4	17.5	1.2	1.3		
60	12.6	0.3	0.6	0.1	0.4	21.4	2.3	2.1		
61	11.7	0.7	1.2	1.1		15.7	1.1	0.9		
62	5.3	0.4	0.6	1.0	1.1					
63	3.3	0.7	0.4	0.7	0.7					

Values indicate stimulation indices (see Fig 1). Underlined values indicate stimulation indices ≥2.0 for responses to the T1, T2, TH4.1 and P18 peptides. Peptides were tested at 2.5 μM and FLU as in Fig. 1. For IL-2 production, all supernatants were tested at 5 serial dilutions in quadruplicate. Although means for 1:4 dilution are shown, they represent results of 20 assays wells. Proliferation assays (day 7) were done in triplicate wells (3×10^5 PBL per well).

* The first 21 donors were HIV^+ individuals whose PBL generated IL-2 in response to FLU. The donor designated LW is a laboratory worker who was accidently infected with $HIV\text{-}1_{IIIB}$.

† The second 21 donors were HIV^+ individuals whose PBL did not generate IL-2 in responses to FLU.

‡ Donors 43-63 were HIV^- individuals whose PBL were used as controls.

PBL from the other Walter Reed Stage 1 patient (583 $CD4^+$ cells mm^{-3}) (Fig. 1*c*) failed to respond either to FLU or to any of the four peptides. Thus, we have identified two types of HIV^+ individuals, one who responds to FLU and to the four HIV synthetic peptides, and another who does not respond to FLU or to any of the four peptides tested. Using the experimental design shown in Fig. 1, we tested PBL from an additional 40 HIV^+ individuals and 20 HIV^- control donors (Table 1). We also tested all donors for response to tetanus toxoid and found a complete concordance with their responsiveness to FLU.

Of the 21 HIV^+ donors who responded to FLU by IL-2 production, only 5 of the 17 tested responded with T-cell proliferation against FLU. In contrast, all of the 19 normal controls responded by T-cell proliferation against FLU. This result indicates that capacity for T-cell proliferation is lost earlier in HIV infection than for IL-2 production. This observation may in part account for earlier failures to detect responses to HIV antigens[10]. To be sure that the failure to observe proliferation despite a positive IL-2 response was not simply due to a difference in the kinetics of the T-cell proliferative response between the HIV^+ individuals and the controls, we investigated the kinetics in three HIV^+ individuals and one control. The control was positive for IL-2 production on day 7, for proliferation on days 5, 7, and 9 and weakly responsive on day 11 of culture. By contrast, the three HIV^+ donors were all negative for proliferative response on days 5, 7, 9, and 11, despite being reproducibly positive for IL-2 production in the same experiment (data not shown). Also, five donors who were unresponsive to FLU by both assays remained negative at all four time points. Therefore, the difference is a qualitative defect in proliferative response and not just a difference in kinetics. Follow up studies of these donors may indicate whether the difference observed at this early time point after infection is of prognostic significance.

Among the 21 HIV donors who responded to FLU, 14 responded to T1 and 12 responded to T2. PBL from 6 of 10 of these same donors tested with the TH4.1 peptide responded, and 5 of 10 tested with peptide P18 responded. Only two of the 21 HIV^+ donors who were unresponsive to FLU responded to any of the four peptides. None of the 21 HIV^-/FLU^+ control donors responded to any of the peptides. Two of the HIV^+ donors who responded to FLU and to one or more of the HIV

TABLE 2 Lack of correlation between HLA class II antigen expression in HIV^+/FLU^+ donors and IL-2 response to HIV synthetic peptides

Donor	HLA antigens		IL-2 response to peptide			
	DR	DQ	T1	T2	TH4.1	P18
363	13, 52	1	+	+	+	+
471	2, 8, 52	1	+	+	−	+
149	4, 52, 53	3	+	−	NT	NT
146	3, 7	2, 3	+	+	NT	NT
318	3, 52	1	−	−	NT	NT
320	3, 4, 52	?	−	−	NT	NT
326	1, 8	1	+	−	NT	NT
382	2, 3, 52	?	+	+	NT	NT
331	11, 13, 52	1, 3	−	+	NT	NT
108	1, 9, 52, 53	1, 3	+	−	NT	NT
425	1, 4, 52, 53	1	+	+	NT	NT
772	8, 13, 52	1	+	+	+	−
LW	1, 2, 52	1	+	−	+	−

NT, not tested.

peptides by IL-2 production were available for re-testing one to three months later, and were found to be reproducibly positive against the same antigens. One HIV^+ donor who showed no response to FLU or to any of the peptides remained negative to all of the antigens when retested one year later. PBL from 13 of the 21 HIV^+ donors who responded to FLU were typed for HLA. In this small sample, no correlation was noted between the expression of a particular HLA class I or class II antigen and ability to respond to any of the synthetic peptides (Table 2).

The individuals who responded to the synthetic peptides were two-to-three times as frequent among HIV^+/FLU^+ donors as among all HIV^+ donors (Table 3). All donors, irrespective of their HIV status, were responsive to HLA alloantigens, which is indicative of their ability to respond to some antigenic stimulus by IL-2 production. It has recently been demonstrated that T-helper cell responses by human PBL to FLU require MHC self-restricted $CD4^+$ T-helper cells whereas the response to alloantigens can use either the $CD4^+$ or $CD8^+$ pathway of T-helper cell activity[7] (C.S. Via, G. Tsokos and G.M.S., manuscript in preparation). Also, 40–50% of asymptomatic HIV^+ individuals and AIDS patients have a selective defect in CD4-mediated but not in CD8-mediated T-helper cell function (M. C. *et al.*, submitted). Therefore, almost all individuals responsive to one or more of the HIV synthetic peptides also responded to FLU, probably because this group of 21 of the 42 HIV^+ donors tested retained intact T-helper cell function to a CD4-dependent antigen. In contrast, 20 of the 21 patients unresponsive to FLU may also have failed to respond to the peptides because they had lost responsiveness to any CD4-dependent antigens. Thus, it is important when testing any HIV^+ individuals for T-helper cell responses to HIV antigens to establish whether these patients have retained an intact CD4 T-helper cell pathway.

Of the 21 FLU-responsive HIV^+ donors, 18 responded to at least one of the four T-cell epitopes. Of the 10 donors we were able to test with all four peptides, nine responded to at least one peptide. Therefore, these four helper T-cell epitopes are sufficient to elicit responses in 85–90% of an HLA-diverse group of patients. For the purposes of vaccine development, it has been feared that many T-cell epitopes would be necessary to cover most or all of the outbred human population with extensive HLA polymorphism. But the present results are much encouraging: only a few selected epitopes may be sufficient. □

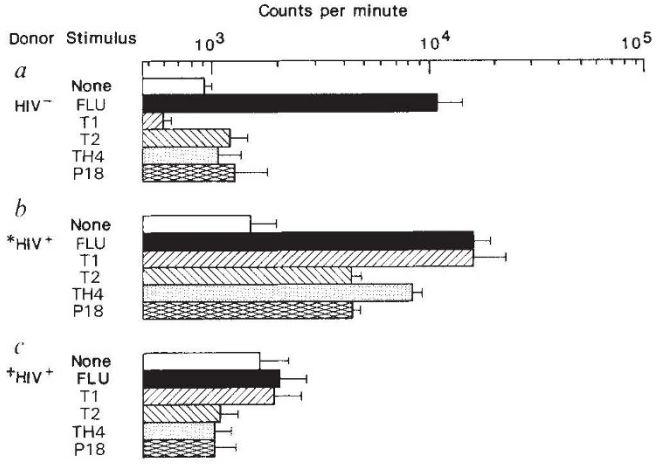

FIG. 1 IL-2 production by PBL from an HIV seronegative (*a*) and two HIV seropositive Walter Reed Stage 1 patients (*b* and *c*).
METHOD. PBL were unstimulated, or stimulated with influenza A virus (A/Hong Kong RX73, H3N2, grown in chicken eggs, final culture dilution 1:1,000) or with the HIV synthetic peptides T1, T2, TH4 or P18 at 2.5 μM. EnvT1 and T2 correspond to amino-acid residues 428–443 and 112–124 of gp120 (IIIB isolate) respectively[1]. Peptide TH4.1 corresponds to residues 834–848 of gp160 (P. Hale *et al.*, submitted). P18, corresponding to residues 315–329 of gp160 (IIIB), is a major epitope for murine anti-HIV cytolytic effector cells[11]. PBL (3×10^6) were cultured in 2 ml RPMI-1640 medium supplemented with 5% pooled AB^+ human serum for 7 d in the presence of anti-TAC (IL-2 receptor p55 chain) monoclonal antibody (to prevent IL-2 consumption). Supernatants of these cultures were collected, and five twofold dilutions of the supernatants were added to cultures of the IL-2-dependent CTLL cell line. Twenty-four hours later, the stimulated CTLL cultures were pulsed with [^{3}H]thymidine; thymidine incorporation was determined 18 h later, and is expressed in counts per minute. The data points shown in Fig. 1 and Table 1 are for a culture supernatant dilution of 1:4. We have used a stimulation index (ratio of counts per minute in stimulated cultures to that in unstimulated cultures) of greater than 2.0 as an indication of positive response. For key, see Table 1.

TABLE 3 Positive T-helper cell responses to the four HIV synthetic peptides as a function of ability to respond to influenza A virus

Donor category	IL-2 production				Proliferation			
	T1	T2	TH4.1	P18	T1	T2	TH4.1	P18
All HIV^+	14/42 (33%)	12/42 (29%)	6/29 (21%)	6/29 (21%)	4/35 (11%)	4/35 (11%)	1/22 (5%)	1/22 (5%)
HIV^+, FLU^+	14/21 (67%)	12/21 (57%)	6/10 (60%)	5/10 (50%)	4/17 (24%)	3/17 (18%)	1/6 (17%)	1/6 (17%)

Fractions and per cent (in parentheses) given for each donor category. All of the HIV seronegative control donors responded to FLU, but none of them responded to any of the four HIV synthetic peptides.

Received 18 November 1988; accepted 19 April 1989.

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ACKNOWLEDGEMENTS. We thank Drs Steven M. Banks and David Alling (NIAID, NIH) for statistical analysis of the data for possible HLA associations, and Ms Judy Kress for preparing the manuscript. The opinions expressed herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Air Force or the Department of Defense.

An engineered poliovirus chimaera elicits broadly reactive HIV-1 neutralizing antibodies

David J. Evans*, Jane McKeating†, Janet M. Meredith*, Karen L. Burke*, Kersi Katrak‡, Ann John‡, Morag Ferguson‡, Philip D. Minor‡, Robin A. Weiss† & Jeffrey W. Almond*

* Department of Microbiology, University of Reading, London Road, Reading RG1 5AQ, UK
† Institute of Cancer Research, Chester Beatty Laboratories, Fulham Road, London SW3 6JB, UK
‡ National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QR, UK

THE Sabin type 1 vaccine strain of poliovirus is probably the safest and most successful live-attenuated vaccine virus used in humans. Its widespread use since the early 1960s has contributed significantly to the virtual eradication of poliomyelitis in developed countries. We have reported previously the construction of an intertypic antigen chimaera of poliovirus, based on the Sabin 1 strain, and proposed that this virus could be modified to express on its surface antigenic determinants from other pathogens[1]. We describe here the construction and characterization of a poliovirus