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Mapping of IgG subclass and T-cell epitopes on HIV proteins by synthetic peptides

T. MATHIESEN,*†‡ P.-A. BROLIDEN,*‡§ J. ROSEN,¶ & B. WAHREN* *Department of Virology, National Bacteriological Laboratory, †Department of Neurosurgery, ‡Department of Virology, §Deparment of Immunology, Karolinska Institute, Stockholm, Sweden and ¶Johnson & Johnson Biotechnology Center, La Jolla, California, U.S.A.

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SUMMARY

Fifteen amino acid peptides, sequentially overlapping by 10 amino acids, were synthesized on the basis of the HTLV-III sequences of the gag and env proteins. They were used as antigens in IgG subclass ELISAs and T-cell stimulation assays. Sera and cells were obtained from 30 asymptomatic, HIV-infected homosexuals. In all subclasses reactivity was found to parts of the gag protein, while IgG1 dominated anti-env peptide responses. It was possible to delineate peptides showing restricted IgG subclass responses that were dominated by either IgG1, 2, 3 or 4. A negative correlation was generally observed between B-cell and T-cell reactivity, but a T-cell and B-cell co-operation was suggested by the response to two IgG1-restricted peptides. The IgG3-dominated epitopes were present in peptides previously known to be amphipathic and capable of T-cell stimulation. The analysis of subclass-restricted responses on the peptide level will assist the understanding of the subclass expression *in vivo*, since the peptide mapping approximates the delineation of a subclass-restricted response at the level of single epitopes.

INTRODUCTION

In vaccine design and in the study of viral antigen drift, it is helpful to know which antigenic determinants on a virus will react with antibodies or effector cells that can destroy the virus. Peptide technology has permitted experiments with omission and substitution peptides (Geysen, Meloen & Barteling, 1984; Geysen, Mason & Rodda 1988) that identify IgG epitopes as groups of four to five peptide residues that interact with the respective IgG paratopes. Unlike B-cell epitopes, most T-cell stimulating sites are composed of stretches of peptides (Streicher *et al.*, 1982; DeLisi & Berzofsky 1985; Margalit *et al.*, 1987). Human B- and T-cell reactivities seem to be directed to different regions in an immune response against, for example, hepatitis B virus (Milich *et al.*, 1986) and HIV (Wahren *et al.*, 1988). The reactivities may overlap, however (Steward *et al.*, 1988). The determination of the nucleotide sequence of HTLV-III (Ratner *et al.*, 1985) allows the synthesis of peptides mimicking parts of the HIV-encoded proteins. Such peptides have been used to study the serological responses to HIV (Gnann *et al.*, 1987; Chiodi *et al.*, 1987; Rusche *et al.*, 1988; Wahren *et al.*, 1988) and the T-cell responses to HIV (Cease *et al.*, 1987; Wahren *et al.*, 1988). The IgG subclass response to whole HIV appears to show an IgG1 and 3 restriction (Sundqvist *et al.*, 1986; Khalife *et al.*, 1987; Mathiesen *et al.*, 1988c). Such a restriction may have implications for virus clearance, since the different subclasses seem to have different capacities to participate in the anti-viral defence (Mathiesen *et al.*, 1988b; Bindon *et al.*, 1988). Recently the IgG response to an immunodominant gp41-derived peptide (amino acid 589–609) was found to show an IgG1 and 2 restriction (Chiodi *et al.*, 1988).

A 15 amino acid peptide can contain only few epitopes. This study was undertaken to see whether an IgG subclass restricted response could be defined on the level of a single peptide. The single peptide response would approximate the subclass responses to a single epitope. The IgG subclass responses are largely regulated by T cells (Rosenberg, 1982; Mosman *et al.*, 1986). For this reason, the IgG subclass responses and T-cell stimulatory responses were compared to determine whether any of the IgG subclasses would be correlated with T-cell reactivity.

IgG subclass-reactive epitopes were identified by ELISAs employing sequential overlapping 15 amino acid peptides as antigens. The relation between IgG subclass reactivities was compared to the T-cell reactive sites in individual patients, as published in a separate study on the T-cell stimulatory responses in HIV-infected patients (Wahren *et al.*, 1988).

Correspondence: Dr T. Mathiesen, Dept. of Virology, National Bacteriological Laboratory, S-105-21, Stockholm, Sweden.

MATERIALS AND METHODS

Patients

Sera and heparinized whole blood samples were obtained from a total of 30 asymptomatic, HIV-infected homosexual men 24–49 years old. Peripheral blood monouclear cells and sera from healthy adults served as controls. The IgG subclass patterns and

T-cell reactivities were investigated in three groups: 10 patients for analysis of the gag region, 10 patients for the COOH-terminal half of gp120 and 10 patients for gp41. The results were recorded as the frequencies of reactive sites, sums of optical density (OD) values for the different peptides, and reactive IgG1–4 and T-cell epitopes in individual patients. For individual peptides, the sign test (Siegel & Castellan, 1988) was used to delineate peptides showing a preference for reactivity with a specific IgG subclass. The term 'subclass restriction' is used to denote such a subclass preference.

The number of patients showing IgG reactivity to each peptide was recorded. For each subclass, presence in the anti-peptide response was scored as (+), absence as (−). Thus a peptide showing an IgG1+2 response in two patients and an isolated IgG1 response in five patients will be recorded as an IgG1 epitope.

Peptides

These were synthesized according to the solid-phase method of Merrifield (1963) modified by Houghten (1985). This method yields 60–90% pure peptides (Houghten, 1985) that are applicable in ELISA detection of specific antibodies (Geysen *et al.*, 1988). Pentadecapeptides, sequentially overlapping by 10 amino acids, were synthesized on the basis of the HTLV-IIIB, clone B10 sequence (Ratner *et al.*, 1985) of gag proteins (peptides 5–32/p17/; 31–78/p24/; 80–102/p15/; amino acids 1–492), the COOH-terminal half of gp120 (peptides C42–92; amino acids 249–513) and gp41 (peptides 223–287; amino acids, 522–856).

For IgG subclass ELISAs, microwell plates (Nunc Immunoplate I, Nunc, Odense, Denmark) were coated with 1 μg of peptide/microwell. Duplicate serum samples were assayed in dilutions 1:100 and 1:1000 in ELISA buffer for 105 min at 37°. The buffers used and the subsequent steps have been described for anti-HIV IgG1–4 ELISAs (Mathiesen *et al.*, 1988c). Briefly, mouse monoclonal antibodies against IgG1 (clone NL16; Seward Laboratories, London, U.K., diluted 1:2000), IgG1 (clone HP6014; Center for Disease Control, Atlanta, GA, diluted 1:1000), IgG3 (clone ZG4; Seward, diluted 1:2000) and IgG4 (clone RJ4; Seward, diluted 1:800) were used to bind to human sera and subsequently detected by HRPO-conjugated anti-mouse Ig (Dako, Copenhagen, Denmark). A colour reaction was obtained with ortho-phenylene diamine (OPD) and the OD at 490 nm was recorded. Blanks and HIV seronegative controls were included in all plates. Sera giving $\geq$ three times the mean OD of negative controls (always more than mean + 3 SD) were scored as positive for the respective IgG subclass and peptide.

T-cell stimulation studies

These were carried out as described elsewhere (Wahren *et al.*, 1988). Briefly, mononuclear cells from heparinized whole blood samples were separated by density gradient centrifugation (Isopaque–Ficoll; Pharmacia, Uppsala, Sweden). 2×10^5 mononuclear cells in RPMI-1640 medium (Gibco, Flow Laboratories, Irvine, Renfrewshire, U.K.; supplemented with 10% HIV antibody-negative AB+ human serum) were assayed in triplicate in sterile microplates (Falcon 3040, Becton-Dickinson Labware, Oxnard, CA) with wells peptide-coated for the IgG testing. The microplates were incubated at 37° in 5·3% CO_2 for 6 days. One μCi of [^{3}H]methyl thymidine was added 24 hr before harvest in a semi-automatic cell harvester (Skatron, Lierbyen, Norway). Radioactivity was averaged over the triplicate cultures and the ratios between peptide-coated wells and control wells were calculated. A ratio ≥ 3 was considered positive.

RESULTS

Gag proteins (p17, p24 and p15)

Figure 1 shows the added OD values of IgG1–4 and the added T-cell reactivity to peptides. Figure 1 and Table 1 show that IgG1 and 3, singly or in combination, were frequently reactive with the gag peptides. The individual subclass patterns and T-cell reactivity were recorded. Regions with apparent preferences for one or two IgG subclasses are shown in Table 2. IgG1 reactivity was found to a majority of gag peptides. The OD values were 0·6–1·2 except for the 373–412 region, where higher values were detected. A reactivity with 80–90% of tested sera was found around amino acids 373–412. IgG2 reactivity was much less common but 80% of the patients reacted to the region bounded by amino acids 88–102. The OD values were lower then for IgG1. The IgG3 responses to these peptides and the OD values were similar to those obtained for IgG1. A difference was that IgG3 dominated the responses to regions including amino

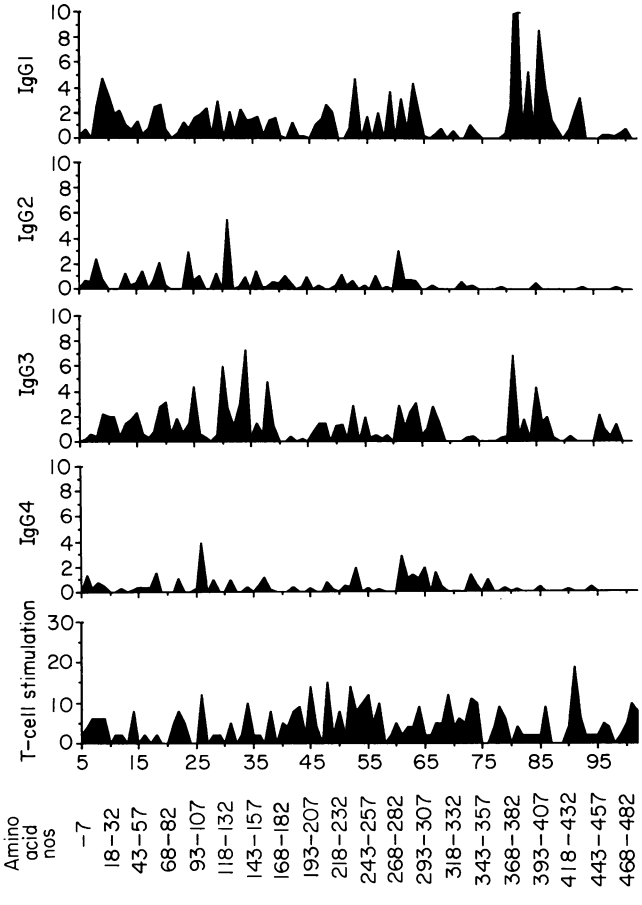

Figure 1. The added absorbance values of IgG1–4 and the added T-cell reactivity of 10 sera investigated for reactivity to the sequentially overlapping peptides 5–102 representing amino amids 1–492 of the gag protein. Only values >3 SD over the mean value of negative controls were included.

Table 1. Combinations of IgG subclasses to the different HIV proteins

	The added patient/peptide reactivities to the respective sets of gag, gp120 and gp41 peptides			
	gag	gp120	gp120	gp41
IgG1	74 (31%)	30 (43%)	28* (74%)	70 (66%)
IgG2	3 (1%)	—		9 (8%)
IgG3	48 (20%)	33 (48%)	4* (11%)	2 (2%)
IgG4	17 (7%)	2 (3%)	2* (5%)	3 (3%)
IgG1+2	9 (4%)	—		10 (9%)
IgG1+3	34 (14%)	4 (6%)	4* (11%)	6 (6%)
IgG1+4	4 (2%)	—		4 (4%)
≥IgG sc.	51 (21%)	—		2 (2%)

All patients showed IgG1 to all three groups of proteins.

Responses showing characteristic patterns are underlined. Summary data such as these cannot be subjected to a meaningful statistical analysis. The percentage figures show percentage of a certain subclass distribution to one peptide of the added positive reactions for 10 patients to all peptides derived from either gag, gp120 or gp41.

* Denotes values obtained for gp120 after exclusion of one patient, who showed a deviant IgG3-dominated response to the gp120 peptides.

acids 93–107, 118–132 and 128–152, while IgG1 dominated the responses to regions including amino acids 373–432. IgG4 reactivity was infrequent and mostly found to amino acids 98–112, where an isolated IgG4 response was found in four patients and an IgG1+4 in three.

Scattered T-cell reactivity was distributed over the peptides. It was negatively correlated with IgG reactivity of any subclass. T-cell responses were associated with IgG1 epitopes in five instances and with IgG1+2 in one, IgG3 in one, IgG1+3 in one, and IgG1+4 in one. IgG1 was over-represented in the rare event that simultaneous T-cell and B-cell reactivities were detected to one peptide (Table 1).

gp120

Figure 2 shows the added OD values of IgG1–4 and the added T-cell reactivity to tested gp120 peptides. IgG1 reactivity was found to regions of amino acids 304–318, 324–343 in 40–60% and to amino acids 489–503 in 90% of tested patients. IgG3 reactivity was found almost exclusively in one patient who showed reactivity in the IgG3 subclass to 29 of the 59 gp120 peptides tested (the patient was not IgG1 deficient, since IgG1 responses were encountered to two of the gp120 peptides). IgG2 and 4 reactivities were negligible. The major T-cell reactivity was detected in the COOH-terminal. IgG1 antibodies (Table 3) were the only IgG antibodies reactivies with amino acids 304–318,

Table 2. IgG subclass and T-cell reactive epitopes with gag peptides

Peptide	Amino acid no.	No. of positive sera	Dominant subclass	Mean OD of positive samples	*P*-value (binomial of $P=0{\cdot}5$)	No. of T+B cell responses	Common other IgG subclasses	Epitope characteristics*
gag								
p17								
9–11	13–37	8	IgG1	0·81	<0·10		—	Philic
18	58–72	5	IgG1	0·58	<0·05		4 varying	A
24	88–102	6	IgG2	0·98	<0·05		4 (IgG1, IgG3)	
25	93–107	6	IgG3	0·88	<0·10		2 (1+3)	A, T
26	98–112	7	IgG4	0·60	<0·05		3 (IgG1)	
29	113–127	6	IgG1	0·52	<0·05		2 (IgG2)	
30	118–132	7	IgG3	0·87	<0·05	1T+3	—	A, T
p24								
32	128–142	9	IgG3	0·58	<0·25		—	
33	133–147	5	IgG3	1·10	n.s.		1 and/or 2	A, T
34	138–152	9	IgG3	0·92	<0·25	1T+1	1 (IgG1)	
38	158–172	6	IgG1+3 or 4		n.s.		6 varying	
53	233–247	7	IgG1	0·74	<0·05		4 varying	
57	253–267	5	IgG1	1·0	n.s.		3 varying	
59	263–277	5	IgG1	1·10	<0·10		—	Phobic
p15								
81–	373–383	7	IgG1	2·0	<0·05		5/8 (IgG1+3)	
85–86	393–413	10	IgG1	0·83	>0·05		2/7 (IgG1+3)	Philic
91	423–437	5				5T		A, T

Peptides reacting with more than 50% of the sera are shown. A statistical analysis was made with the sign test. The number of patients showing IgG reactivity to each peptide was recorded. For each subclass, presence in the anti-peptide response was scored as (+), absence as (−). Thus a peptide showing an IgG1+3 response in two patients and an isolated IgG1 response in five patients will be recorded as an IgG1 epitope; the number of multisubclass reactive sera is also given.

* T, T-cell activity; A, amphipathicity; phobic, hydrophobic region; philic, hydrophilic region.

Table 3. IgG subclass and T-cell reactive gp120 epitopes

Peptide	Amino acid no.	No. of positive sera	Dominant subclass or T cell	Mean OD value of positve	*P*-value (binomial of $P=0{\cdot}5$)	No. of T+B cell responses	Common other IgG subclasses	Epitope characteristics*
env								
gp120								
C53	304–318	9	IgG1	0·77	<0·05		—	Philic
C54–55	309–328	4				4T	—	T, A
C57	324–338	5	IgG1	0·96	<0·05		—	
C71–72	394–413					4T		T, A
C84	459–473					4T		T, A
C90	489–503	9	IgG1	1·28	<0·05	4T+1		Intermediate
C92	499–512					5T		

* T, T-cell activity; A, amphipathicity; phobic, hydrophobic region; philic, hydrophilic region.

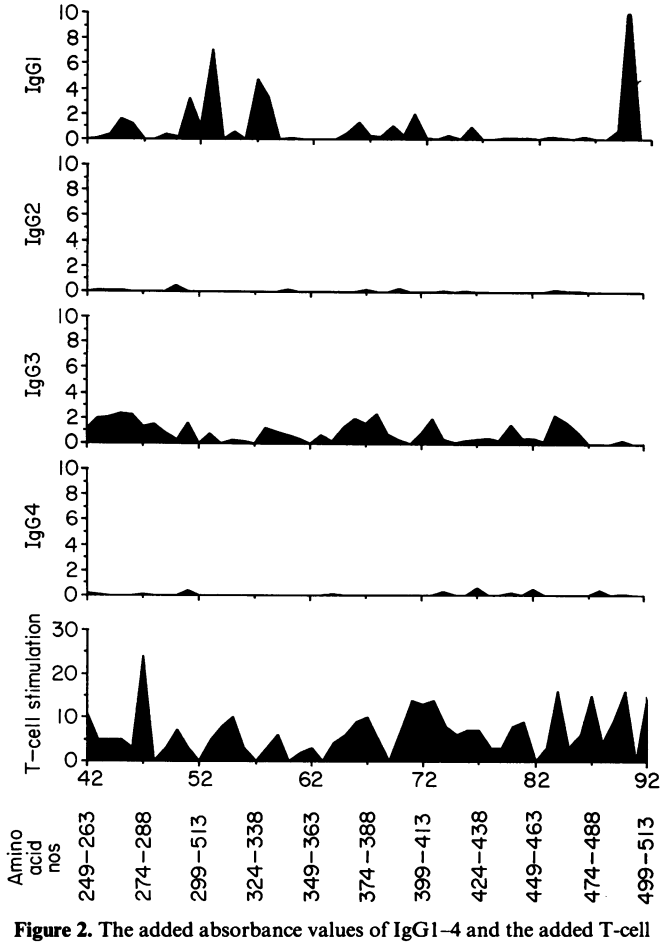

Figure 2. The added absorbance values of IgG1–4 and the added T-cell reactivity of 10 sera investigated for reactivity to the sequentially overlapping peptides 42–92 representing amino acids 249–513 of gp120. Only values >3 SD over the mean value of negative controls were included.

324–338, and 489–503. T-cell reactive was more common to gp120 than to gp41 and gag (Table 4). As with gag, T-cell stimulation was detected in the absence of IgG subclass reactivity. A combination of T-cell reactivity and IgG1 reactivity was noted to the region of amino acids 489–503 in four patients.

Table 4. Combination of simultaneous reactive T cells and IgG subclasses to the different proteins

	The added patient/peptide reactivities to the respective sets of gag, gp120 and gp41 peptides		
	gag	gp120	gp41
T cell only	30	56	36
T+IgG1	5	5	7
T+IgG3	1	1	—
T+1+2	1	—	—
T+1+3	1	—	—
T+1+4	1	—	—

The T-cell responses were usually detected in the absence of IgG responses to the same peptides. When simultaneous T- and B-cell reactivity was detected, IgG1 was over-represented.

gp41

Figure 3 shows the added OD values of IgG1–4 and the added T-cell reactivity to peptides. The IgG response was dominated by IgG1 both in the frequencies of reactive patients and in OD values recorded (Table 5). The main reactive regions were the immunodominant amino acids 582–616, 617–636, and 792–811. The highest OD values were recorded for amino acids 582–616. IgG1 was the prevailing subclass to these regions (Table 5). Distinct IgG2 responses, alone or in combination with IgG1, were detected to amino acids 597–611 and 617–631. IgG1 or 2 were thus the only IgG subclasses found to react with amino acids 597–611. Low positive OD values were demonstrated for IgG3 or IgG4 in a few patients. Scattered T-cell reactivity was present in regions non-reactive with IgG. Three patients showed a simultaneous IgG1 and T-cell rectivity to amino acids 587–601 and four to 837–856 in the COOH-end.

DISCUSSION

The present study demonstrates the feasibility of peptide mapping to detect IgG subclass-restricted anti-peptide responses. These responses were interpreted as being directed to

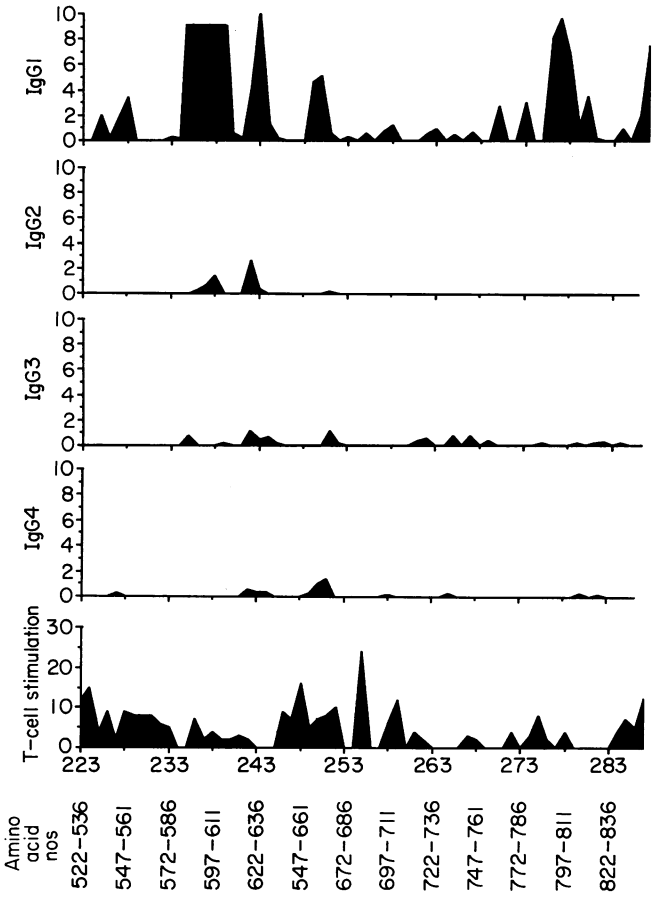

Figure 3. The added absorbance values of IgG1–4 and the added T-cell reactivity of 10 sera investigated for reactivity to the sequentially overlapping peptides 223–287 representing amino acids 522–856 of gp41. Only values > 3 SD over the mean value of negative controls were included.

continuous epitopes present in the peptides. The peptide mapping approximates the delineation of a subclass-restricted response at the level of a single epitope. It must, however, be kept in mind that even a 15 amino acid peptide may contain several epitopes. The peptides used in the present study were derived from gag proteins (p17, p14 and p15), the COOH-half of gp120 and from gp41. Sundqvist *et al.* (1986) demonstrated a tendency to IgG1+3 restriction to HIV. Khalife *et al.* (1987) utilized immunoblotting to demonstrate an IgG1 restriction to gp120 and gp41, while IgG1, 3 and 4 were reactive with gag proteins. A similar pattern of IgG1 dominance to envelope proteins and IgG1, 3 and 4 to core proteins has been demonstrated for mumps (Linde, Granström & Örvell, 1987) and HSV (Ljungman *et al.*, 1988). The HIV peptide findings corroborate the higher frequency of IgG3 in an anti-gag response than in responses to env proteins. The peptide ELISA is probably more sensitive than immunoblotting and thus enabled the detection of IgG3 epitopes in all patients investigated and IgG2 and/or 4 epitopes in a majority. Such a higher sensitivity with peptide mapping compared to Western blotting was also noted by Broliden *et al.* (1988) in a follow-up study on 20 patients with HIV infection. They also found IgG3 to be reactive mainly with gag-derived peptides. The peptide assays might detect epitopes not presented by the denatured antigens in the blotting procedures. An additional explanation to the high frequences of anti-peptide IgG2–4 may be that our patients were asymptomatic: a progressive HIV infection is correlated with the decrease of IgG subclasses other than IgG1 (Sundqvist *et al.*, 1986; Mathiesen *et al.*, 1988c). A definite advantage of the present method is that the peptide ELISA permits an analysis of anti-peptide IgG2, while the available anti-human IgG2 antibodies are, according to Khalife *et al.* (1987), unsuitable for staining immunoblots.

Immunodominant peptides were identified as peptides to which more than 50% of the sera showed an IgG reactivity of any subclass. For each peptide, the IgG subclass expression was analysed by the sign test to investigate whether the peptides showed an IgG subclass preference. In the present context, the subclass restriction is thus defined in terms of occurrence of one defined subclass. It was necessary to analyse isolated subclass responses rather than combinations of subclasses. One reason for dividing the observations into two categories (presence or absence of each subclass) instead of 16 (all possible combination of the four subclasses) is to maximize the possibility of detecting differences in the subclass responses. The possible false negative results would thus not invalidate the interpretation of a

Table 5. IgG subclass and T-cell reactive gp41 epitopes

Peptide	Amino acid no.	No. of reactive sera	Dominant subclass	Mean OD value for positive samples	*P*-value (binomial of = *P*0·5)	No. of T+B cell responses	Common other IgG subclasses	Epitope characteristics*
env								
gp41								
235	582–596	9	IgG1	1·67	<0·05		2(1+3)	
236	587–601	8	IgG1	2·0	<0·05	3T+1	2(1+2)	
238	597–611	10	IgG1	2·0	<0·05		3(1+2)	Phobic
239	602–616	9	IgG1	1·6	<0·05		No other	Phobic
242–243	617–636	7	IgG1	1·4	<0·1		Varying	
249–250	652–671	6	IgG1	0·87	<0·05		2(1+4)	
277–278	792–811	5	IgG1	1·84	<0·1		1(1+4)	
(287	842–856		IgG1	1·7	NS	4T+1		

* T, T-cell activity; A, amphipathicity; phobic, hydrophobic region; philic, hydrophilic region.

'restricted response', as the restriction is understood as a preference of expression of a particular subclass.

It was possible to delineate subclass-restricted anti-peptide IgG responses. IgG1 was the most common IgG subclass, reactive with 30 peptides. This was hardly surprising, since the bulk of antiviral immunoglobulins is found in the IgG1 fraction. For the gag peptides, the highest IgG1 reactivities were detected in amino acids 13–37, rich in glycine, arginine and lysine and thereby hydrophilic with a high segmental flexibility; IgG2, 3 and 4 epitopes were found in the hydrophilic COOH-terminal of p17; the second highest IgG1 and 3 reactivities were found in the hydrophilic C-terminal of p24. It was possible to detect peptides with preferences for not only IgG1 but also IgG2, 3 or 4. For example, the peptide 26 (amino acids 98–112) showed an isolated IgG4 reactivity in four out of 10 patients and an IgG1 + 4 response in three out of 10.

Env peptides were almost exclusively IgG1 restricted. The subclass responses to some region of known functional or antigenic importance were of special interest. One of these regions, amino acids 296–331, is the putative loop region in gp120 that appears to mediate HIV neutralization (Ho *et al.*, 1987; Goudsmit *et al.*, 1988; Rusche *et al.*, 1988). This region was IgG1 restricted. Ljunggren *et al.* (1988) showed HIV neutralization and antibody-dependent cellular cytotoxicity (ADCC) in the IgG1 but not in the IgG3 fractions of sera separated by Staphylococcal-protein A chromatography. An absence of IgG3 reacting with the neutralizing epitopes of HIV would explain the apparent deviation from earlier reports claiming IgG3 to be a functionally important anti-viral antibody in other viral diseases (Beck, 1981; Mathiesen *et al.*, 1988b). A second IgG1-restricted epitope was detected in the hydrophilic COOH-terminal end of gp120, corresponding to the SP22 peptide (Palker *et al.*, 1988). Of the antigenic sites on gp41, amino acids 587–617 correspond to a conserved, highly immunogenic region (Gnann *et al.*, 1987; Chiodi *et al.*, 1987). In the present study, this region showed an IgG1 restriction and an absence of IgG3 response. High titres of IgG2 were detected to this region in three patients. The material is too small to support a claim of an exclusive IgG1 or IgG1 + 2 response but the results do agree with those of Chiodi *et al.* (1988), who detected an IgG1 + 2-dominated response to this region in a study of 100 HIV-infected patients. Interestingly, one human monoclonal antibody directed to this region (Banapour *et al.*, 1987) is of the IgG2 subclass.

An exception to the rule of IgG1 restriction to env peptides, was the finding of an IgG3-restricted reactivity to 29 gp120 peptides in a single patient. Animal studies show that different mouse strains may respond in a subclass-restricted manner to the same antigens, but that the pattern of restriction may differ between the strains (Slack, 1987). Similarly, humans with an absence of the IgG1 heavy-chain encoding gene segments show high titres of anti-measles IgG3, while normal humans show an IgG1 response to the same antigen (Mathiesen *et al.*, 1988a). This finding underlines the influence of the genetic characteristics of a responding immune system; the subclass-restricted responses detected in animal models with inbred mice cannot be compared with those detected in humans without accounting for the multitude of factors that can influence an individual immune response. An inter-individual variation in the subclass responses was obvious in the entire study. The IgG2 and/or 4 responses were directed to several peptides in the patients showing anti-peptide IgG2 or 4 reactivity, while some patients did not show IgG2 or 4 reactivity to any peptide at all. This contrasted to IgG1 and 3 responses, which were detected in all patients.

A second objective of the present study was to compare T-cell reactive sites to B-cell subclass epitopes in the same patient in order to see whether any particular subclass would correlate with T-cell stimulation. Wahren *et al.* (1988) described the T-cell stimulating peptides to which these patients reacted as mainly amphipathic (DeLisi & Berzofsky, 1985). The T-cell proliferation assay mainly measures the activity of T-helper cells (Ljungman, Sundqvist & Wahren, 1985) but it is not known whether any particular T-cell subset will respond preferentially. T-cell proliferative responses were detected against many peptides but almost exclusively in areas not binding IgG. Peptides with simultaneous T- and B-cell reactivity were detected, and showed a preference for IgG1. The finding was clearest for the env amino acids 489–503 and for amino acids 842–856. The T-cell and IgG1 reactivity with the same peptide may suggest that T- and B-cells may co-operate in an immune response to identical epitopes or epitopes situated close to each other. It is also possible that the few responses to the same peptides are due to a random statistical process and is not relevant to the human immune response. *In vitro*, T-cell derived lymphokines influence subclass switches (Snapper, Finkelman & Paul, 1988). It is possible that the T-cell population stimulated in the present assay belongs to a population mainly synthesizing lymphokines with an IgG1-inducing effect, as suggested for murine helper cells by Mosman *et al.* (1986). Another interesting correlation between IgG3 responses and T-cell responses was detected. The IgG3 gag peptides amino acids 93–107, 128–142 and 118–132, the latter an IgG3 site, did not elicit strong T-cell responses in these patients but they compromise amphipathic regions with a T-cell stimulatory capacity (Wahren *et al.*, 1988). Thus, a negative correlation prevailed between B-cell and T-cell reactivity. It cannot be ruled out that the tendency to simultaneous T- and B-cell responses on some occasions may reflect a T- and B-cell interaction. This study included relatively few patients but was sufficient to identify immunogenical regions with specific IgG subclass responses. The analysis of subclass-restricted responses on the peptide level provides a mean of studying the multifactoral subclass expression *in vivo*. The usually discrepant T- and B-cell responses are a rationale for including several selected peptides in a putative vaccine.

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