Clin Exp Immunol 1993; 94:17-20

HIV induces deletion of T cell receptor variable gene product-specific T cells

A. S. BANSAL*†, L. M. GREEN*, S. H. KHOO‡, R. S. H. PUMPHREY*, M. R. HAENEY† & B. K. MANDAL‡ *Department of Immunology, St Mary's Hospital, Manchester, †Department of Immunology, Hope Hospital, Salford, and ‡Department of Infectious Disease, Monsall Hospital, Manchester, UK

(Accepted for publication 7 July 1993)

SUMMARY

Using a flow cytometric method, $CD4^+$, $CD8^+$, $\alpha\beta$ TCR^+ and TCR variable region gene product (TVRGP)-specific T cells were analysed in healthy heterosexual males (HHeM), HIV-seronegative homosexual males (SNHM), asymptomatic seropositive homosexual males (ASPH) and homosexual males with AIDS who were either well (AIDS-A), or unwell in hospital (AIDS-B). Total $CD4^+$ and $CD8^+$ T cell numbers were similar in HHeM and SNHM. $CD4^+$ T cells were significantly reduced in ASPH relative to both HHeM and SNHM and in AIDS-A and AIDS-B relative to SNHM. TVRGP-specific T cells expressed as a percentage of TCR $\alpha\beta^+$ cells showed no significant difference in HHeM, SNHM and AIDS-B. The proportion of $\alpha\beta^+$ cells expressing the Vβ5.1, Vβ12 and Vα2 gene product (GP) was, however, significantly reduced in ASPH and AIDS-B relative to HHeM, SNHM and AIDS-A. Possible causes of TVRGP-specific T cell deletion are discussed.

Keywords HIV T cell receptor flow cytometry

INTRODUCTION

The reason for the decline in CD4 T cell numbers accompanying the progression of HIV infection is currently unclear. Recent reports have suggested an importance for alloimmune mechanisms [1] and superantigen activity [2,3] in the T cell depletion of HIV infection. In the former, sequence homologies between HIV and MHC molecules are believed to induce a chronic graft-*versus*-host (GVH)-like disease with attendant T cell activation, B cell hyperactivity and impaired antigen-specific responses [4]. In the latter, HIV is considered to encode a superantigen. This binds MHC class II proteins on antigen-presenting cells to T cells bearing specific variable regions on the β chain of the T cell receptor (TCR). The interaction produces initial T cell activation followed by deletion. Both alloreactive and superantigen mechanisms also obviate the need for actual HIV infection of T cells for T cell elimination. Furthermore, both mechanisms may induce a state of functional T cell paralysis. This would produce an impairment of cell-mediated immunity disproportionate to the actual numbers of HIV-infected T cells.

The phenotypic assessment of TCR variable region expression is limited by the number of MoAbs available. Recent estimates for the number of Vβ families range from 20 to 30, whilst those for the Vα families ranges from 50 to 60. Using a panel of seven TCR variable region-specific MoAbs we have found a selective decline in T cells bearing the Vβ5.1, Vβ12 and Vα2 gene product (GP) in asymptomatic HIV-seropositive homosexual males (ASPH) relative to healthy controls [5]. In order to investigate this further we analysed TCR variable region gene product (TVRGP) expression in healthy heterosexual males (HHeM, $n=13$), HIV-seronegative homosexual males (SNHM, $n=9$), ASPH ($n=16$) and CDC group IV homosexual males who were either well and attended as out-patients (AIDS-A, $n=11$) or were unwell as in-patients in hospital (AIDS-B, $n=9$). This allowed the comparison TCR V region expression in healthy males with a group of homosexual males exposed to the similar cofactors but differing in their HIV status and stage of infection.

Correspondence: Dr M. R. Haeney, Clinical Sciences Building, Department of Immunology, Hope Hospital, Eccles Old Road, Salford M6 8HD, UK.

PATIENTS AND METHODS

TCR $\alpha\beta$ and variable region expression was assessed by a flow cytometric method using the 'Identi T: kit' (Diversi-T ab TCR, T Cell Sciences) as previously described [5]. The results are expressed as percentages of $\alpha\beta$ TCR^+ cells. Analysis of T cell CD4 and CD8 expression utilized flow cytometry and directly conjugated (MoAbs) T4 and T8 (Coulter). HIV status was checked by an ELISA method and confirmed by Western blotting. HIV^+ patients were staged according to the CDC classification 1986 [6]. The Mann–Whitney *U*-test was used in all statistical analysis. Patients in AIDS-B required hospitalization for the following reasons: *Pneumocystis carinii* pneumonia ($n=3$), cytomegalovirus retinitis ($n=3$), severe microsporidial or cryptosporidial diarrhoea ($n=1$ each) and pulmonary tuberculosis ($n=1$).

Table 1. Total CD4 and CD8 counts in the patient groups studied

Patient group	Total CD4 count ($\times 10^9/l$)	Total CD8 count ($\times 10^9/l$)
HHeM ($n=13$)	1·05 (0·71–1·25)	0·70 (0·45–0·91)
SNHM ($n=9$)	1·21 (0·81–1·50)	0·78 (0·39–0·85)
ASPH ($n=16$)	0·40 (0·35–0·45)	1·06 (0·81–1·21)
AIDS-A ($n=11$)	0·11 (0·07–0·20)	0·87 (0·36–1·30)
AIDS-B ($n=9$)	0·02 (0·01–0·07)	0·42 (0·26–0·48)

Data expressed as median (interquartile range). HHeM, Healthy heterosexual males; SNHM, seronegative homosexual males; ASPH, asymptomatic seropositive homosexual males; AIDS-A, out-patients; AIDS-B, in-patients.

CD4 count: SNHM *versus* ASPH, $P<0·002$; ASPH *versus* AIDS-A, $P<0·0003$; AIDS-A *versus* AIDS-B, $P<0·05$. CD8 count: SNHM *versus* ASPH, $P<0·005$.

RESULTS

Table 1 details the results of CD4 and CD8 T cell analysis. SNHM had similar results to HHeM. Relative to SNHM, ASPH showed a significant decline in the total CD4 count. This was accompanied by a significant increase in the total CD8 count. With HIV progression from asymptomatic disease to AIDS-A and AIDS-B, reductions in the total CD4 and CD8 counts were evident. The total CD8 count was significantly higher in AIDS-A relative to HHeM and SNHM. In AIDS-B both the CD4 and CD8 populations showed a further proportionally equivalent decline.

Table 2 records the results of Vβ5.3 (using MoAbs βV5a and βV5b, clones ICI and W112 respectively), Vβ6.7 and Vβ8 GP expression; no significant difference was evident in the percentage of these T cells in each of the groups analysed.

Figure 1 summarizes the results of Vβ5.1, Vβ12 and Vα2 GP expression and the results of statistical analysis. As with the CD4 and CD8 analyses, SNHM had virtually identical results to HHeM. Significantly lower proportions of Vβ5.1, Vβ12 and Vα2 GP bearing T cells were noted in ASPH compared with HHeM and SNHM. With HIV progression from asymptomatic disease to AIDS-A the pattern of T cell variable gene usage showed no significant change, despite reductions in both CD4 and CD8 T cells. The development of acute illness in AIDS-B produced proportionally equivalent decreases in both CD4 and CD8 T cells, but an increase in the proportion of T cells expressing the Vβ5.1, Vβ12 and Vα2 GP. Consequently the pattern of TVRGP expression showed no significant difference in HHeM, SNHM and AIDS-B.

DISCUSSION

No single explanation is able to account for the selective decline in T cell numbers accompanying HIV infection. HIV superantigen activity, alloimmune mechanisms, gp120-induced programmed cell death, autoimmune destruction and differences in T cell susceptibility to HIV infection have all received close support at one time or another.

We have previously found a selective decline in the numbers of T cells bearing specific TVRGP in ASPH relative to healthy controls. Our current findings confirm the importance of HIV in the etiology of this change, as SNHM had an almost identical distribution of TVRGP-specific T cells as that observed in HHeM. Although our patient numbers are small, this suggests that the cofactors considered important in the immune attrition of advancing HIV infection are unlikely on their own to have significant superantigen activity.

Grunewald *et al.* have reported biased TVRGP expression in CD4 and CD8 T cells [7]. This confirmed significantly skewed reactivity of the Vβ5.1, Vβ6.7, Vβ8 and Vβ12 GP expression in favour of the CD4 population, while Vβ5.3 and Vα2 GP expression was equivalent in both CD4 and CD8 T cells. It is therefore possible that changes in TVRGP expression observed with HIV infection and its progression may be the result of changes in the CD4/CD8 subsets. We have observed a significant depression in Vβ5.1, Vβ12 and Vα2 GP expression in ASPH relative to both HHeM and SNHM. As the percentage of Vβ6.7 and Vβ8 GP bearing T cells remained unchanged whilst that of the Vα2 GP bearing T cells was depressed, we believe that our results suggest a specific deletion of these cells rather than a change resulting from increased CD8 T cells. This pattern of T cell deletion appears to be a feature of early HIV infection, as progression from asymptomatic disease to AIDS-A produces no change in the proportions of any of the TVRGP-specific T cells. Vβ5.1, Vβ12 and Vα2 bearing T cells which survive the initial deletion during asymptomatic disease appear to be as susceptible to further deletion as other TVRGP-specific T cells. In the case of Vα2 GP bearing T cells we have recorded a mean value of 4·02% in HHeM and SNHM. This is reduced to less than 1% in ASPH and AIDS-A, suggesting that both CD4 and CD8 T cells are suffering deletion. The cause of this deletion is unclear, as superantigens binding MHC class II to the variable region of the TCR α chain have not, as yet, been reported. Furthermore, the deletion appears to involve both CD4 and CD8 T cells. This would require superantigens binding MHC class I to CD8 T cells as well as MHC class II to CD4 T cells, and alloreactivity to involve sequence homologies of gp120/41 to both classes of MHC protein. Whilst this is still possible, our findings do cast some doubt on the singular importance of either of these hypotheses. A simpler explanation would be that continual HIV mutation in lymphoid tissue induces selective T cell migration and sequestration. This would be compatible with the highly infectious passive carriage of HIV by follicular dendritic cells and macrophages in secondary lymphoid tissue [8,9].

The rise to normal levels in the proportion of Vβ5.1, Vβ12 and Vα2 GP bearing T cells in acutely unwell AIDS patients (AIDS-B) suffering further loss of CD4 and CD8 T cells, suggests accelerated loss of T cells bearing TVRGP other than those assessed. This may simply represent migration of these lymphocytes from the blood to the sites of active infection/inflammation, and analysis of the same patients following recovery would be highly informative. Our results do not, however, allow clarification of which subset, i.e. CD4 or CD8, contributes to most of this change. If HIV superantigen activity or gp120/41 alloreactivity were to be important in this depletion, then significant changes in critical areas of the involved HIV proteins would be required to allow suitable interactions.

Our findings show a selective decline in the proportions of TCR VR-specific T cells in asymptomatic HIV infection and in patients with AIDS who are well at the time of analysis. Meyaard *et al.* [10] describe increased programmed cell death

Table 2. Vβ5.3, Vβ6.7 and Vβ8 gene product-positive cells as a percentage of $\alpha\beta^+$ T cells in all the groups analysed

Patient group	TVRGP+T cells as % of $\alpha\beta^+$ cells			
	Vβ5.3+*	Vβ5.3+†	Vβ6.7+	Vβ8+
HHeM ($n=13$)	2·7 (2·4–3·0)	1·1 (0·8–1·2)	2·2 (1·8–3·5)	3·9 (3·6–4·3)
SNHM ($n=9$)	2·5 (2·3–2·9)	0·9 (0·7–1·0)	2·2 (1·7–4·1)	3·7 (2·7–5·0)
ASPH ($n=16$)	2·7 (2·0–3·2)	0·9 (0·7–1·2)	1·5 (0·8–2·1)	3·3 (2·3–4·3)
AIDS-A ($n=11$)	2·5 (1·5–2·8)	1·3 (0·6–1·7)	2·3 (1·6–4·3)	3·0 (2·3–3·7)
AIDS-B ($n=9$)	3·1 (1·7–4·1)	1·1 (0·7–1·6)	1·6 (1·2–2·6)	3·9 (1·5–5·4)

TVRGP, T cell receptor variable region gene product; HHeM, healthy heterosexual males; SNHM, seronegative homosexual males; ASPH, asymptomatic seropositive homosexual males; AIDS-A, out-patients; AIDS-B, in-patients.

* MoAb Vβ5a (clone ICI).

† MoAb Vβ5b (clone W112). Data expressed as median (interquartile range). No significant difference in T cell TVRGP expression between any of the patient groups.

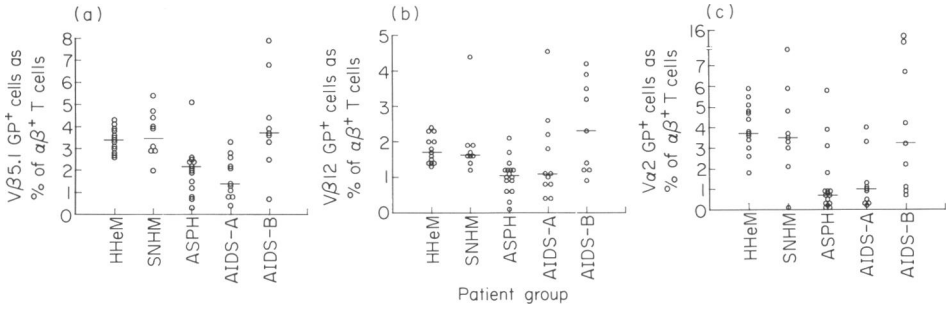

Fig. 1. Vβ5.1 (a), Vβ12 (b) and Vα2 (c) expression in all patient groups. No statistical difference in T cell receptor variable region gene product (TVRGP) expression between healthy heterosexual males (HHeM), seronegative homosexual males (SNHM) and AIDS-B (in patients) and between asymptomatic seropositive homosexual males (ASPH) and AIDS-A (out-patients). Percentage of Vβ5.1, Vβ12 and Vα2 T cells significantly higher in HHeM, SNHM and AIDS-B relative to ASPH and AIDS-A ($P<0·01$ to $<0·001$ except for Vβ5.1 in SNHM *versus* ASPH, $P<0·02$; Vβ12 in AIDS-A *versus* AIDS-B, $P<0·05$; Vα2 in AIDS-A *versus* AIDS-B, $P<0·03$).

(PCD) of both CD4 and CD8 T cells in ASPH. The fact that three out of seven TVRGP-specific MoAbs have shown such a change suggests that differences in susceptibility to deletion of TVRGP-specific T cells are quite widespread. Using a polymerase chain reaction method Imberti *et al.* [2] also found selective reductions of several TCR VR-specific T cells. As activated [11] and memory [12] $CD4^+$ T cells are more susceptible to HIV infection, replication and gp120-mediated PCD it is possible that our results may simply reflect the antigen specificity of the deleted T cells, i.e. the deleted T cells are those reactive against HIV or other commonly encountered antigens. Thus, HIV would appear to be important for the peripheral blood depletion of Vβ5.1, Vβ12 and Vα2 GP bearing T cells, whilst other microbial agents may be important for the depletion of non-Vβ5.1, Vβ12 and Vα2 GP bearing T cells when patients with AIDS become unwell. Differences in T cell susceptibility to HIV infection have been noted by Laurence *et al.* [3]; Vβ12 T cell lines yielded up to 100-fold more p24 gag gene product than Vβ6.7a lines, and cells from patients positive for gp120 were enriched in the Vβ12 subpopulation. Since the pattern of exposure to infectious agents is different between homosexual males, intravenous drug abusers and haemophiliacs, the variation in TVRGP-specific proportions in these groups may be quite illuminating.

Our results confirm the importance of HIV in mediating the selective depletion of peripheral blood V gene-specific T cells in ASPH and AIDS-A. This depletion almost certainly affects both CD4 and CD8 T cells and several V gene-specific T cells are affected. A variety of mechanisms may be important in this respect. These would include HIV superantigen activity, gp120/41-induced alloreactivity, differential T cell activation or infection, and selective lymph node migration. During progression from asymptomatic disease to AIDS-A, all T cells appear equally susceptible to deletion. This may reflect equivalent degrees of activation by circulating cytokines, cross-linked CD4-bound gp120 or gp120 multimers. With acute illness (AIDS-B) a greater susceptibility to depletion is evident amongst TVRGP-specific T cells different from those affected in early HIV infection. The mechanisms for this change are unclear, and may reflect differences in microbial T cell activation and thus susceptibility to PCD or selective lymph node migration in response to infection/inflammation. It is likely that several mechanisms may be operative at each stage of HIV infection.

REFERENCES

1 Dalgleish AG, Wilson S, Gompels M *et al.* T-cell receptor variable gene products and early HIV-1 infection. Lancet 1992; **339**:824–8.

2 Imberti L, Sottini A, Bettinardi A. Selective depletion in HIV infection of T cells that bear specific T cell receptor Vβ sequences. Science 1991; **254**:860–2.

3 Laurence J, Hodtsev AS, Posnett DN. Superantigen implicated in dependence of HIV-1 replication in T cells on TCR Vβ expression. Nature 1992; **358**:255–9.

4 Habeshaw J, Hounsell E, Dalgleish A. Does the HIV envelope induce a chronic graft-*versus*-host-like disease? Immunol Today 1992; **13**:207–14.

5 Bansal AS, Green L, Pumphrey RSH, Mandal BK. T cells, V genes and HIV. Lancet 1992; **339**:1604.

6 Selick RM, Jaffe HW, Soloman SL, Curran JW. CDC definition of AIDS. N Engl J Med 1986; **315**:761.

7 Grunewald J, Janson CH, Wigzell H. Biased expression of individual T cell receptor V gene segments in $CD4^+$ and $CD8^+$ human peripheral blood T lymphocytes. Eur J Immunol 1991; **21**:819–22.

8 Pantaleo G, Graziosi C, Demarest JF *et al.* HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature 1993; **362**:355–8.

9 Embretson J, Zupancic M, Ribas JL *et al.* Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature 1993; **362**:359–62.

10 Meyaard L, Otto SA, Jonker RR *et al.* Programmed death of cells in HIV-1 infection. Science 1992; **257**:217–9.

11 Groux H, Torpier G, Monte D *et al.* Activation-induced death by apoptosis in $CD4^+$ T cells from Human Immunodeficiency Virus-infected asymptomatic patients. J Exp Med 1992; **175**:331–40.

12 Schnittman SM, Lane HC, Greenhouse J *et al.* Preferential infection of $CD4^+$ memory T cells by human immunodeficiency virus type 1: evidence for a role in the selective T-cell functional defects observed in infected individuals. Proc Natl Acad Sci USA 1990; **87**:6058–62.