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γδ T cells in rhesus monkeys and their response to simian immunodeficiency virus (SIV) infection

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SUMMARY

Recent reports of the increase in peripheral blood γδ T cells in HIV^+ patients prompted us to examine the γδ T cell system in rhesus monkeys (*Macaca mulatta*) and the responses of these cells to SIV infection. Our results reveal differences in the γδ T cell subset composition and their expression of CD8 in the peripheral blood of monkeys and humans. The outgrowth of simian γδ T cells in response to Daudi cells is similar to that in humans, but the exposure to IL-2 stimulates preferentially the simian Vδ1 subset rather than the Vγ9/Vδ2 subset as found in humans. Upon SIV infection of the monkeys, we observed a transient increase of the percentage of total γδ T cell and the Vγ9 subset. γδ T cells from infected animals also express more activation markers such as CD69, CD44 and the memory marker CD45RO. However, they respond to a lesser degree to Daudi or IL-2 stimulation in the outgrowth experiments compared with uninfected animals, although the subset composition of total γδ T cells is similar in infected and uninfected animals. The results clearly indicate that γδ T cells in rhesus monkeys are influenced by SIV infection. The detailed analysis of the γδ T cell response to SIV infection can serve as a model for understanding human γδ T cell responses to HIV infections.

Keywords γδ T cell T cell subset rhesus monkey simian immunodeficiency virus

INTRODUCTION

The numbers of γδ T cells and their activities are substantially changed during various pathophysiological situations [1]. For example, the percentage and absolute numbers of Vδ1 T cells in the human peripheral blood are increased in many adult patients as well as children infected with HIV [2,3]. The percentages of Vγ9/Vδ2 T cells are lower in HIV-infected individuals compared with control individuals, whereas the percentages of Vδ1 T cells coexpressing Vγ9 and Vγ2,3,4 are significantly increased [4,5]. This expansion of Vδ1 T cells does not appear to be clonal due to the absence of restricted Vδ1–Jδ1 junctional sequences associated only with disease [4,5]. SIV infection of rhesus monkeys (*Macaca mulatta*) is the best animal model for human AIDS. The recently reported [2–5] changes in the γδ T cell representation in peripheral blood mononuclear cell (PBMC) preparations from AIDS patients prompted us to investigate the γδ T cell system in rhesus monkeys to see if parallel observations can be made and analysed. This analysis could provide a better understanding of how the activity of γδ T cells influences the course of infection or *vice versa*. Since very little is known about γδ T cells in rhesus monkeys, we first examined some of the potential similarities and/or differences that may exist between γδ T cells in monkeys and humans. As a first assessment of the influence of infection on γδ T cell reactivities, we compared the phenotypes and responsiveness of γδ T cells from uninfected monkeys and monkeys infected with SIV using various γδ T cell stimuli.

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MATERIALS AND METHODS

Animals

Thirteen age-matched rhesus monkeys were randomly selected from the colony and were confirmed to be SIV^- by polymerase chain reaction (PCR) and virus isolation before infection. Each was given a 20 animal infectious dose of SIVmac251 intravenously. All monkeys were positive for virus isolation at all time points examined after infection. Peripheral blood was collected by venipuncture from SIV-infected or uninfected control monkeys.

Cells and cell lines

PBMC were isolated from blood of monkeys by density gradient centrifugation using Ficoll–Hypaque (Sigma Chemical

Co., St Louis, MO). Daudi cells were passaged twice weekly in RPMI 1640 medium containing 10% fetal bovine serum (FBS; Intergen, Purchase, NY), 100 U/ml penicillin (Mediatech, Washington, DC), 100 μg/ml streptomycin (Mediatech), and 2 mM L-glutamine (Mediatech).

Flow cytometric analysis of fresh and cultured lymphocytes

Freshly isolated or cultured PBMC (5×10^5) were stained with the FITC-conjugated pan γδ T cell MoAB TCRδ1, or FITC-conjugated δV1a, δV2a and γV2a with specificity for Vδ1, Vδ2 and Vγ9, respectively (T Cell Diagnostics, Cambridge, MA). For examining the expression of activation markers on these cells, PE-conjugated CD8 MoAb (Sigma) or non-conjugated MoAbs followed by goat anti-mouse antibody conjugated to PE (GAMIg PE) (Boehringer Mannheim, Indianapolis, IN) were added. The MoAbs used were specific for the IL-2 receptor (T Cell Diagnostics), CD69 (Becton Dickinson, Mountain View, CA), CD44 and CD45RO, generously provided by Dr. E. Clark (University of Washington, Seattle, WA) [6]. IgG1 isotype control antibodies (Sigma) were also included. After 30 min on ice, cells were washed with ice-cold PBS, fixed in 1% paraformaldehyde and analysed on a FACScan flow cytometer (Becton Dickinson).

Daudi and IL-2 stimulation of PBMC

PBMC were cultured at 10^6 cells/ml in RPMI 1640 medium containing 10% FBS (Intergen), 100 U/ml penicillin (Mediatech), 100 μg/ml streptomycin (Mediatech), and 2 mM L-glutamine (Mediatech). In the Daudi expanded cultures, 2×10^5 irradiated (120 Gy) Daudi cells/ml and 50 U/ml of IL-2 (Biological Modifiers Response Program, NCI, Frederick, MD) were supplemented. In the IL-2 expanded cultures, 200 U/ml of IL-2 were supplemented. Half the media of the cultures were aspirated on day 6 and supplemented with new media containing 50 or 200 U/ml IL-2 for the Daudi or IL-2 expanded cultures, respectively. Cell counts and the percentages of cells were assessed on day 11.

Statistical analysis

All statistical comparisons were performed by the Wilcoxon signed rank test [7] and the two-tailed P values are reported. Differences with P values < 0.05 were considered significant.

RESULTS

Phenotype of γδ T cells

Similar to humans [8], the percentage of γδ T cells varies in individual monkeys and ranges from < 1% to > 10% (Fig. 1) [9]. However, the γδ T cell subset distribution in monkeys is different from that in humans. In contrast to man, where the major subset of γδ T cells is $V\gamma9^+ V\delta2^+$ [10], simian Vδ1 T cells consistently outnumber Vδ2 and the number of Vγ9 T cells is very variable. The Vγ9/Vδ2 T cells are rarely detectable by dual-colour flow cytometry, and the cells are mostly singly Vγ9 and Vδ1-positive (data not shown). When the same monkeys were infected intravenously with SIV, there was a transient increase in the percentage of γδ T cells 2 and 3 weeks after infection in most monkeys, and the levels decreased to the pre-infection levels 6 weeks after infection. The changes in percentages also reflected the changes in absolute numbers. The transient increase and subsequent decrease of γδ levels were statistically significant. The changes in levels of the various γδ T cell subsets throughout infection were not significant, except for the Vγ9 T cells, where the transient increase by 2 and 3 weeks post-infection mirrored that of γδ T cells and was significant.

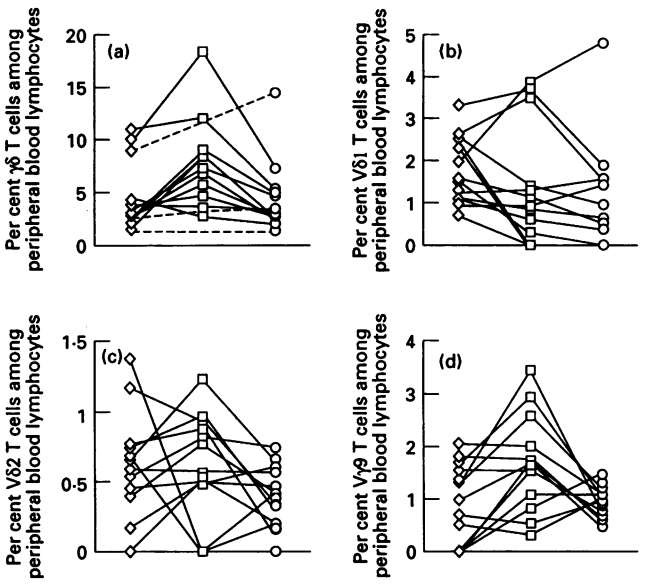

Fig. 1. Percentages of γδ (a), Vδ1 (b), Vδ2 (c) and Vγ9 (d) in the peripheral blood lymphocytes (PBL) of monkeys before infection (◇), at 2–3 weeks post-infection (□) and 6 weeks post-infection (○). Each symbol represents one monkey and each line connects different time points of the same monkey. The change in γδ percentage from pre-infection to 2–3 weeks post-infection is significant ($P = 0.0137$). The change from 2–3 weeks to 6 weeks post-infection is significant ($P = 0.0020$). All changes in Vδ1 and Vδ2 percentages are not significant. The change in Vγ9 percentage is significant from pre-infection to 2–3 weeks post-infection ($P = 0.0327$), but not significant from 2–3 weeks to 6 weeks post-infection.

γδ T cells were next examined for the expression of various markers. Most expressed CD8, and this did not change with infection (Fig. 2). This is in contrast to what is known in humans, where most γδ T cells are CD4 and CD8-negative [11]. The majority of γδ T cells from uninfected animals did not express p55, the inducible α chain of the IL-2 receptor on their surface. They also did not express the memory marker CD45RO and the early T cell activation antigen, CD69, but most were $CD44^+$ (Fig. 3). However, a significantly higher percentage of γδ T cells from infected animals expressed CD45RO and CD69, suggesting previous *in vivo* activation.

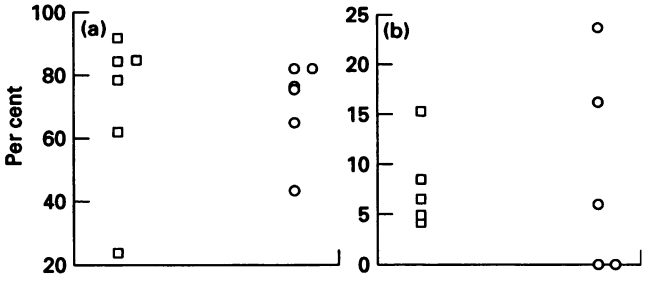

Fig. 2. Expression of activation markers on γδ T cells analysed by dual-colour flow cytometry. Coexpression of CD8 (a) and IL-2R (b) on γδ T cells before (□) and after (○) SIV infection. $n = 6$ for CD8 and $n = 5$ for IL-2R.

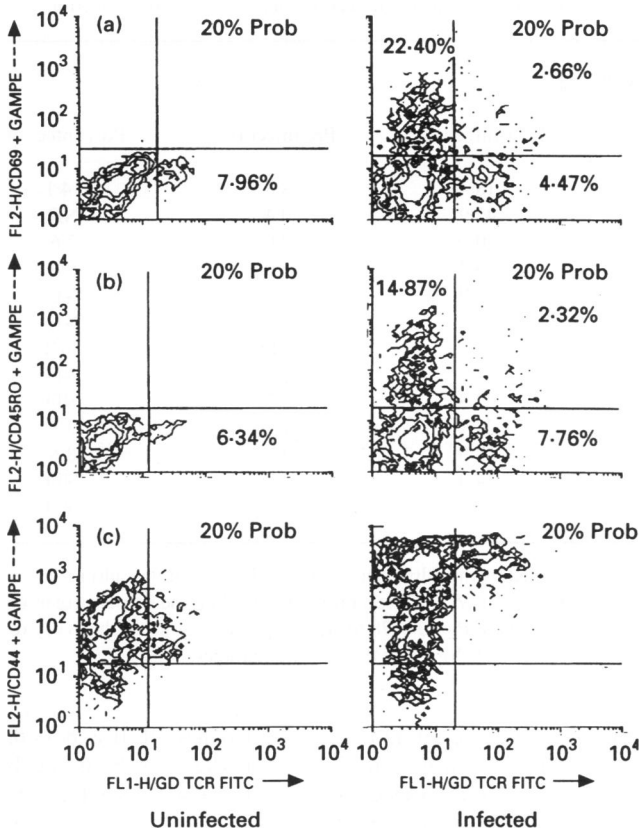

Fig. 3. Expression of CD69 (a), CD45RO (b) and CD44 (c) on γδ T cells. A representative contour plot each from an uninfected and infected animal is shown for comparison. The mean of γδ T cells expressing CD69 for uninfected monkeys is 0%, while that of infected animals is 46·05% ($n = 4$). The mean for CD45RO expression is 3·5% for uninfected animals ($n = 4$) and 23·4% for infected animals ($n = 7$).

They also expressed a higher density of CD44 on their surface. Since CD44 is an adhesion marker, it is possible that these γδ T cells were activated to home into particular tissue sites.

Expansion profile of γδ T cells

Besides the phenotypic changes that occurred upon SIV infection, γδ T cells from infected animals might respond differently to various stimuli than those from uninfected animals, since their γδ T cells appeared to have been activated. PBMC isolated from the peripheral blood of the animals were cultured in the presence of irradiated Daudi cells with 50 U/ml IL-2 or with 200 U/ml IL-2 alone for 11 days (Table 1). We found that the percentages and absolute numbers of γδ T cells were increased in all cultures. The γδ subset distribution of these cells was also examined by flow cytometry using various T cell receptor (TCR) MoAbs. We observed that the cultures stimulated with irradiated Daudi cells always contained more Vγ9/Vδ2 T cells than those stimulated only with IL-2, where Vγ9/Vδ2 T cells were virtually non-existent (Table 1). Both types of cultures contained substantial percentages of $V\delta1^+$ $V\gamma9^-$ T cells, which were slightly higher in the IL-2-stimulated cultures. Nevertheless, both types of cultures were still abundant in γδ T cells belonging to undefined subsets. This type of expansion profile is very different from similar cultures of human γδ T cells, where an outgrowth of Vγ9/Vδ2 T cells appears to be independent of the particular stimuli [12,13].

γδ T cells from both uninfected and infected animals were expanded in percentage and numbers similarly in response to both stimuli examined, and the subset composition of γδ T cells remained unchanged. Since the input percentages of γδ T cells and the various subsets at the start of the cultures were different in the uninfected and infected animals, we also calculated the stimulation indices of γδ, Vγ9/Vδ2 and Vδ1 T cells (Tables 2 and 3). In most animals, the γδ T cell expansions induced by the Daudi or IL-2 stimuli were significantly diminished after infection. There were no significant changes in the stimulation indices for the various γδ subsets.

DISCUSSION

Previous reports have demonstrated significant increases in peripheral blood γδ T cells in HIV-infected individuals [2–5]. In this study, we examined certain functions of γδ T cells in control and SIV-infected rhesus monkeys. The same group of animals was used before and after infection to control for potential differences in individual animals in the uninfected and infected groups.

This is the first study concerning the characteristics of simian γδ T cells in SIV-infected animals, and we demonstrate some

Table 1. Comparison of the total percentage of γδ T cells and percentages of γδ T cells expressing Vδ1 or Vγ9/Vδ2 in response to Daudi or IL-2

	Daudi		IL-2	
	Pre-infection	Post-infection	Pre-infection	Post-infection
γδ	47·6 ± 15·9	43·0 ± 14·0	55·1 ± 12·8	54·9 ± 15·7
Vδ1	20·7 ± 10·7†	37·4 ± 9·2*	39·8 ± 10·1†	40·5 ± 5·0*
Vγ9/Vδ2	31·3 ± 19·8†	28·0 ± 13·2	3·4 ± 4·0†	2·3 ± 2·5

γδ percentages were assessed by flow cytometric analysis of total lymphocytes after 11 days of culture with either Daudi and 50 U/ml of IL-2 or 200 U/ml of IL-2. Vδ1 and Vγ9/Vδ2 percentages represent the proportion of γδ T cells expressing these T cell receptors (TCR). All percentages are reported as the mean with s.d. using $(n - 1)$ as the degree of freedom. * $n = 6$; † $n = 12$. All changes were deemed non-significant by the Wilcoxon signed rank test for paired data.

Table 2. Comparison of the stimulation indices of γδ, Vγ9/Vδ2 and Vδ1 T cell in response to Daudi cell before and after SIV infection

	γδ %		Vγ9/Vδ2 %		Vδ1 %	
Monkey	Pre-infection	Post-infection	Pre-infection	Post-infection	Pre-infection	Post-infection
92033	26·0	5·6	15·6	18·4	8·8	14·1
92036	12·9	13·6	58·9	42·9	0·4	ND
92038	6·3	ND	23·6	0	7·0	7·6
92045	21·4	ND	23·5	15·2	4·0	9·0
92052	20·1	12·5	18·4	160·9	4·3	ND
92054	14·5	7·0	120·9	35·1	8·1	ND
92055	ND	9·8	8·2	7·1	ND	26·2
92059	12·2	8·4	14·2	123·2	2·2	ND
92062	6·1	3·8	110·3	43·3	2·7	ND
92068	20·6	3·0	12.1	21·7	1·1	3·8
92073	5·5	ND	91·9	3·9	5·8	8·6
92079	11·1	9·7	12·7	34·0	2·9	ND
91072	19·3	6·3	30·5	12·7	7·0	ND

Stimulation indices are calculated as the final percentage after 11 days in culture divided by the initial percentage at the start of the culture. The γδ percentage represents the percentage of total peripheral blood lymphocytes (PBL) expressing γδ T cell receptor (TCR), while the subset percentage represents the percentage of γδ T cells expressing that particular subset. ND, Not determined. $P = 0{\cdot}0078$ for the γδ percentage using the Wilcoxon signed rank test, as described in Materials and Methods. Data are not significant for the Vγ9/Vδ2, nor the Vδ1 percentages.

Table 3. Comparison of stimulation indices of γδ and Vδ1 T cell in response to IL-2 before and after SIV infection

	γδ %		Vδ1 %	
Monkey	Pre-infection	Post-infection	Pre-infection	Post-infection
92033	31·5	7·6	10·9	21·5
92036	11·6	17·4	1·4	ND
92038	7·2	ND	9·1	7·4
92045	17·1	ND	8·9	24·0
92052	16·9	4·3	11·1	ND
92054	16·4	6·4	12·1	ND
92055	20·2	10·2	8·6	28·5
92059	19·7	8·0	7·3	ND
92062	5·7	5·1	4·8	ND
92068	6·2	3·4	4·6	5·8
92073	15·0	ND	23·4	24·0
92079	8·1	12·3	8·6	ND
91072	26·3	8·3	15·9	ND

Stimulation indices are calculated as the final percentage after 11 days in culture divided by the initial percentage at the start of the culture. The γδ percentage represents the percentage of total peripheral blood lymphocytes (PBL) expressing γδ T cell receptor (TCR), while the subset percentage represents the percentage of γδ T cells expressing that particular subset. ND, Not determined. P value is 0·0371 for the γδ percentage, and is not significant for the Vδ1 percentage.

differences between humans and monkeys. The most important differences are the expression of CD8 by most simian γδ T cells, and the relatively low percentage of simian γδ T cells belonging to the Vγ9/Vδ2 subset. Large proportions of the Vδ1 and other undefined γδ subsets are present in the peripheral blood of monkeys. In humans, most $CD8^+$ γδ T cells and Vδ1 T cells are found among the intestinal intraepithelial lymphocytes [14], perhaps due to their preferential homing in certain tissue sites caused by the recruiting action of cytokines [4]. It is possible that the tissue distribution of rhesus monkey γδ subsets may be influenced by the presence of different homing receptors or a different cytokine milieu in the body.

In contrast to some HIV studies in man, we observed a transient increase in peripheral blood γδ T cells 2 and 3 weeks after the SIV infection of rhesus monkey, but this declined to pre-infection levels within 6 weeks. This early increase is partly due to an increase in Vγ9 T cells expressing undefined Vδ chains. The early increase in γδ T cells during infection is compatible with the idea that γδ T cells are part of front-line defence mechanisms against invading pathogens [15,16]. More γδ T cells from infected animals (compared with controls) expressed CD69 and CD45RO, but not the inducible α chain of the IL-2 receptor. They also expressed more CD44 on their surface, as evident by the higher intensity of staining with the antibody. This suggests a higher degree of activation in SIV-infected animals in comparison with controls. Therefore, it was somewhat surprising to see that γδ T cells from infected animals expanded to a lesser degree when stimulated with Daudi cells or IL-2. It is possible that the viral infection inactivates certain cell types which normally provide help for the expansion of γδ T cells.

In conclusion, although some differences exist between human and simian γδ T cells, the overall similarities between γδ T cells in man and monkey make the rhesus SIV infection a suitable model for analysis of the role of γδ T cells in immunodeficiency virus immunosurveillance.

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