Role of Human Lymphocytes Bearing $\alpha\beta$ and $\gamma\delta$ T-Cell Receptor in Malaria Infection

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Key Words

$\gamma\delta$ T cells · Infectious diseases ·
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Abstract

Objective: To investigate the role of $\gamma\delta$ T cells in the regulation of immune response in *Plasmodium falciparum* infection. **Methods:** $\gamma\delta$ and $\alpha\beta$ T cells were purified from peripheral mononuclear blood cells (PMBC) by positive selection method using monoclonal antibodies. The PMBC were obtained from normal healthy donors. The role of various T cell subsets was investigated by observing their capacity to inhibit the growth of *P. falciparum* parasites. **Results:** By positive selection using monoclonal antibodies we were able to obtain a relatively pure fraction (>95%) of $\gamma\delta$ T cells from PMBC. The parasite growth inhibitory capacity of activated $\gamma\delta$ T cells ranged between 6 and 55%, however, activated $\alpha\beta$ T cells failed to inhibit the growth. It was also observed that the inhibitory capacity of $\gamma\delta$ T cells was dose-dependent and

required physical contact with the organism.

Conclusion: Activated $\gamma\delta$ T cells have the capacity to inhibit the growth of *P. falciparum* parasites and thus may act as a first line of defense in controlling the blood stage infection. The significance of $\gamma\delta$ T cells in influencing various bacterial infections is discussed.

Introduction

It is now well established that T cells play an important role in both the protection and the pathogenesis of patients suffering from infectious diseases. In recent years it has become clear that CD4⁺ T cells can be separated into subsets (TH1/TH2) based upon the repertoire of cytokines produced following stimulation, and that the distinct cytokine profile observed in these subsets determines cell function [1]. The immunological responses that contribute to resistance versus susceptibility to bacterial and parasitic infections seem to depend upon the presence of functionally distinct CD4⁺

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T cells: T helper TH1 and TH2 cells [2]. More than 95% of the T cells express $\alpha\beta$ chains, however, a minor subset (1–5%) of the T cells express γ and δ chains [3]. $\gamma\delta$ T lymphocytes are evenly distributed in peripheral blood and throughout lymphoid tissues. They are the first cells to colonize the thymus before the appearance of $\alpha\beta$ T cells.

With monoclonal antibodies it has now become possible to isolate sub-populations of $\gamma\delta$ T cells and to investigate their functional properties. In peripheral blood $\gamma\delta$ T cells are mainly of two distinct subsets. The major subset expressed V γ 9 and V δ 2 whereas the minor expresses V δ 1-encoded antigen [4]. The majority of $\gamma\delta$ T cells lack CD4 and CD8 expression [5]. CD8, however, can be detected on a fraction of peripheral blood $\gamma\delta$ + T cells. CD4 expression is even less common than CD8 expression on peripheral blood $\gamma\delta$ + T cells [6]. Interestingly, CD4 is readily detected on a fraction of human fetal liver or thymus $\gamma\delta$ + T cells [7]. Between 1 and 17% of splenic lymphocytes is reported to express the $\gamma\delta$ T cell receptor (TCR). $\gamma\delta$ + lymphocytes are preferentially localized in the red pulp of the spleen [8].

The elevation of $\gamma\delta$ T cells in acute and convalescent phase of malaria and their location in the red pulp of the spleen in malaria-infected individuals [8, 9] have led to the suggestion that $\gamma\delta$ T cells may play an important role in controlling blood stage infections. The present study was undertaken to study the effect of $\gamma\delta$ T cells on the growth of *Plasmodium falciparum* malaria parasites.

Materials and Methods

Monoclonal Antibodies

The following monoclonal antibodies were used: anti-TCR-V δ 2 (BB3) and anti-TCR- γ 9 (A13), OKT3 (anti-CD3), OKT4 (anti-CD4) and OKT8 (anti-CD8, Dakopatts, Denmark); anti- $\alpha\beta$ (WT 31) and anti-CD25 from Becton-Dickinson (USA).

Fractionation of $\alpha\beta$ and $\gamma\delta$ T Cells

$\alpha\beta$ and $\gamma\delta$ T-cell-enriched fractions were prepared from the non-adherent cells of the peripheral mononuclear blood cells obtained from healthy donors using monoclonal antibodies. Briefly, adherent cells were depleted by incubation of PMBC in plastic Petri dishes in RPMI containing 10% human serum at 37 °C. Non-adherent cells were recovered by subsequent gentle washing of the Petri dishes in RPMI medium. The non-adherent cells were incubated at 10^7 /ml in RPMI medium for 30 min on ice with 10 μ g/ml each of anti-TCR-V δ 2 (BB3) and anti-TCR- γ 9 (A13) affinity-purified monoclonal antibodies. Excess antibodies were washed away 3 times with cold RPMI containing 10% fetal calf serum. The cells were resuspended at 10^8 /ml in the same medium and added to magnetic beads coated with anti-mouse immunoglobulin antibodies (Dynabeads, Dianova, Munich, Germany) at a concentration of 4 beads/cell. After a further incubation of 30 min on ice, bound cells were removed using a magnetic concentrator (Dianova, Germany) and washed 4 times with cold RPMI and the above procedure repeated on the cells remaining in suspension. The $\alpha\beta$ T cells were recovered from the cell suspension using anti- $\alpha\beta$ (WT31) antibodies and following the same selection procedure as for selection of $\gamma\delta$ T cells.

The phenotype of various effector cell preparations thus obtained was confirmed by immunofluorescent staining of effector cells. Monolayers of cells were prepared on multitest BIO-RAD adhesion slides (BIO-RAD) by fixing in 1% glutaraldehyde in phosphate-buffered saline and air drying. The fixed cells were incubated with indicated monoclonal antibodies (BB3, A13, WT31) with specificity for cell surface markers at predetermined optimal concentrations and visualized with FITC-conjugated goat antimouse immunoglobulin. The cells were examined with a Zeiss fluorescence microscope (Zeiss, Germany) at a magnification of $\times 100$. To determine the frequency of different effector cells a minimum of 2,500 cells were counted. The purity of the $\gamma\delta$ T cells ranged >90%.

Activation of Cells

Highly purified cell fractions (2×10^6 cells/ml) of both $\alpha\beta$ and $\gamma\delta$ T cells were activated identically with phytohemagglutinin (PHA, 2.5 μ g/ml) for 12 h with sonicated parasite crude antigen for 3 days. The cells were then washed and used in the parasite reinvasion inhibition assay.

In vitro Parasite Growth Inhibition Assay

One hundred microlitres of the parasite suspension, 1% infected red blood cells and 100 μ l of washed

Fig. 1. Parasite growth inhibition in vitro of *P. falciparum* malaria parasites with $\gamma\delta$ and $\alpha\beta$ T cell fractions. Different effector:target cell ratios were used to analyse the growth inhibitory capacity of $\alpha\beta$ and $\gamma\delta$ T cells. Both $\alpha\beta$ and $\gamma\delta$ T cells were activated with PHA (2.5 $\mu\text{g/ml}$) for 12 h and with sonicated parasite crude antigen for 3 days. The parasites were maintained in culture for 48 h with effector cells. Percent parasite growth inhibition was calculated as described in 'Materials and Methods'. The data presented is the mean of three experiments done on T cell subsets purified from PMBC of 3 different healthy blood donors.

effector cell suspension at concentrations of 0, 1.25×10^5 , 2.5×10^5 or 5×10^5 cells were plated into 96-well flat-bottomed plates (Flow Laboratories, Irvine, Scotland) in duplicates. The plates were incubated at 37°C in 5% CO_2 in air for 48 h. Thereafter, the cell suspensions were harvested and monolayers of cells prepared on multitest slides by fixing in 1% glutaraldehyde in phosphate-buffered saline and air-dried. The percent of newly infected erythrocytes was analysed by counting infected erythrocytes in 25 fields/well. The percentage inhibition of parasite growth was calculated using the following formula:

$$\% \text{ parasite inhibition} = \frac{\text{Number of ring stages in control} - \text{Number of ring stages in test sample}}{\text{Number of ring stages in control samples}} \times 100$$

Results

Freshly isolated inactivated $\gamma\delta^+$ and $\alpha\beta^+$ did not inhibit the growth of *P. falciparum* parasites (data not shown). However, when these cells were activated with PHA for 12 h or with a crude parasite sonicate for 3 days, the highly enriched $\gamma\delta$ T cells inhibited the growth of malaria parasite. As can be seen in figure 1 there was a dose-dependent inhibition of parasite growth which increased from 6 to 55% using $\gamma\delta$ T cell:infected red blood cell ratios from 1:2 to 4:1, respectively. No significant parasite growth inhibition was observed by activated $\alpha\beta$ T cells regardless of the numbers of cells added to the cultures as shown in figure 1. The parasite growth inhibition seen with the cell-free supernatant obtained from activated $\gamma\delta$ and $\alpha\beta$ T cells was less than 3.5%.

Discussion

T cells have been shown to inhibit in vitro replication of the asexual blood stages of the *P. falciparum* parasite [10]. In agreement with these findings, we found that both PHA- and *P. falciparum* antigen-activated $\gamma\delta$ cells inhibit the growth of the parasite. The parasite growth inhibitory capacity of $\gamma\delta$ T cells ranged from 6 to 55% with highly enriched $\gamma\delta$ T cells. The inhibition was well correlated with the number of $\gamma\delta$ T cells present in the cultures. The observed capacity to inhibit parasite replication in vitro was limited to $\gamma\delta$ T cells since activated $\alpha\beta^+$ T cells failed to inhibit the parasite replication. It can of course be argued that the growth-inhibitory response of the selected $\gamma\delta$ T cells was due to contaminant $\alpha\beta$ ($\text{CD4}^+/\text{CD8}^+$) T cells. However, since this contamination was less than 10% and no growth inhibition was seen with purified activated $\alpha\beta$ T cell fraction, we consider this unlikely.

Parasite growth inhibition was not seen with cell-free supernatants from activated $\alpha\beta$ and $\gamma\delta$ T cells. The role of cytokines and the importance of cell contact in inhibiting the growth of the parasites are being investigated.

During an acute infection with malarial parasite *P. falciparum* circulating $\gamma\delta$ cells display a marked increase in both their relative proportion and absolute numbers [11, 12], which may persist for up to 3–4 months of convalescence [13]. A similar increase in the peripheral $\gamma\delta$ cell fraction, occurring particularly during the period of fever paroxysm, has been described in non-immune patients infected with *Plasmodium vivax* [14]. Furthermore, a profound increase in the splenic $\gamma\delta$ cell population has been reported during malaria infection, with these cells more than doubling their normal proportion to reach 40% of the total lymphocyte population [8]. In humans, the cells activated in vitro by *P. falciparum* are almost exclusively $V\gamma^9\delta^2$ [15, 16] and express an activation marker, CD45RO, suggesting that these cells are repeatedly stimulated. Although it may be assumed that the rapid $\gamma\delta$ T cell response is of importance in the primary immune response to malaria infection, the possible role of these cells in protection and/or pathogenesis of malarial disease is still being investigated.

The potential role of $\gamma\delta$ T cells in the regulation of immune responses is still not very clear. However, it appears that most $\gamma\delta^+$ T cells possess cytolytic activity [17]. Antigens derived from *M. tuberculosis* have been found to induce strong proliferation of $V\gamma^9/V\delta^2$ subset both in vivo and in vitro, suggesting that $\gamma\delta$ T cells might play a role in the immune defense against infection with *M. tuberculosis* in vivo [3, 18]. As a matter of fact, $\gamma\delta$ T cells are present in skin lesions of leprosy patients, however, the $\gamma\delta$ subset seen in the skin lesion is different from that seen in the peripheral

blood [19]. Apart from mycobacteria, it appears that certain other bacteria also stimulate $\gamma\delta^+$ T cells. Among these, *Staphylococcus aureus*, various groups of streptococci, *Salmonella typhimurium*, and *Listeria monocytogenes* were found to induce significant activation of human $\gamma\delta^+$ T cells [20–22], but the role of $\gamma\delta$ T cells in these situations is unclear. In addition, there is clear evidence that $\gamma\delta$ T cells accumulate in the lesions of patients with localized cutaneous leishmaniasis [23]. In chronically infected (susceptible) BALB/c mice, highly elevated numbers (up to 35%) of $\gamma\delta$ T cells were present in the spleen, while in resistant CBA/J mice, the initially increased numbers of $\gamma\delta$ T cells returned to normal levels after lesion resolution. In addition, the blockage of $\gamma\delta$ T cells by injection of anti-TCR- γ/δ monoclonal antibody resulted in exacerbated disease in both strains of mice. Taken together, these data suggest that $\gamma\delta$ T cells might play an important role in at least certain infectious diseases.

In conclusion, our data show that activated $\gamma\delta$ T cells inhibit the growth of *P. falciparum* parasites, while similarly activated $\alpha\beta$ T cells did not. Growth inhibition was not seen with inactivated $\gamma\delta$ and $\alpha\beta$ T cells. Thus, it is likely that $\gamma\delta$ T cells play an important role in the immune defense against malaria infection. The role of cytokines and the molecules involved in the growth inhibition of parasites requires further studies.

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References

- 1 Mosmann TR, Coffman RL: Heterogeneity of cytokine secretion patterns and functions of helper T cells. *Adv Immunol* 1989;46:111–147.
- 2 Mosmann TR, Coffman RL: TH1 and TH2 cells: Different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989;7:145–173.
- 3 Kabelitz D, Pechhold K, Bender A, Wesselborg S, Wesch D, Friese K, Janssen O: Activation and activation-driven death of human $\gamma\delta$ T-cells. *Immunol Rev* 1991;120:71–88.
- 4 Hass W, Tonegawa S: Development and selection of $\gamma\delta$ T cells. *Curr Opin Immunol* 1992;4:147–155.
- 5 Brenner MB, McLean J, Dialynas DP, Strominger JL, Smith JA, Owen FL, Seidman JG, Rosen SIF, Krangel MS: Identification of a putative second T-cell receptor. *Nature* 1986;322:145–149.
- 6 Groh V, Porcelli S, Fabbi M, Lanier LL, Picker LJ, Anderson T, Warnke RA, Bhan AK, Strominger JL, Brenner MB: Human lymphocytes bearing T-cell receptor $\gamma\delta$ are phenotypically diverse and evenly distributed throughout the lymphoid system. *J Exp Med* 1989;169:1277–1294.
- 7 Kabelitz D, Conradt P, Schondelmaier S, Wagner H, Haars R: A novel subset of CD2⁺, CD3/T cell receptor $\alpha\beta$ ⁺ human peripheral blood T cells. *J Exp Med* 1989;170:559.
- 8 Bordessoule D, Gaulard P, Mason DY: Preferential localization of human lymphocytes bearing $\gamma\delta$ T-cell receptors to the red pulp of the spleen. *J Clin Pathol* 1990;43:461–464.
- 9 Phillips S: Effector mechanisms against erythrocytic stage of *Plasmodium*. *Immunol Lett* 1994;41:116–121.
- 10 Fell AH, Currier J, Good MF: Inhibition of *Plasmodium falciparum* growth in vitro by CD4⁺ and CD8⁺ T cells from non-exposed donors. *Parasite Immunol* 1994;16:579–586.
- 11 Ho M, Webster HK, Tongtawe P, Pattanapanyasat K, Weidanz WP: Increased $\gamma\delta$ T cells in acute *Plasmodium falciparum* malaria. *Immunol Lett* 1990;25:139–142.
- 12 Roussilhon C, Agrapart M, Ballet JJ, Benussaan A: T lymphocytes bearing the $\gamma\delta$ T cell receptor in patients with acute *Plasmodium falciparum* malaria. *J Infect Dis* 1990;162:283–285.
- 13 Roussilhon C, Agrapart M, Guglielmi P, Bensussan A, Brasseur P, Ballet JJ: Human TCR $\gamma\delta$ ⁺ lymphocyte response on primary exposure to *P. falciparum*. *Clin Exp Immunol* 1994;95:91–97.
- 14 Perera MK, Carter R, Goonewardene R, Mendis KN: Transient increase in circulating $\gamma\delta$ T cells during *P. vivax* malarial paroxysms. *J Exp Med* 1994;179:311–315.
- 15 Behr C, Dubois P: Preferential expansion of V γ ⁹ V δ ² T cells following stimulation of peripheral blood lymphocytes with extracts of *Plasmodium falciparum*. *Int Immunol* 1992;4:361–366.
- 16 Goodier M, Krause-Jauer M, Langhorne J: Quantitative analysis of the response of human T cell receptor V γ ⁹⁺ cells to *P. falciparum*. *Eur J Immunol* 1992;22:2757–2760.
- 17 De Libero G, Casorati G, Giachino C, Carbonara C, Migone N, Matzinger P, Lanzavecchia A: Selection by two powerful antigens may account for the presence of the major population of human peripheral $\gamma\delta$ ⁺ T-cells. *J Exp Med* 1991;173:1311–1322.
- 18 Kabelitz D, Bender A, Prospero T, Wesselborg S, Janssen O, Pechhold K: The primary response of human $\gamma\delta$ ⁺ T-cells to *Mycobacterium tuberculosis* is restricted to V γ 9-bearing cells. *J Exp Med* 1991;173:1331–1338.
- 19 Modlin RL, Pirmez C, Hofman FM, Torigian V, Uyemura K, Rea TH, Bloom BR, Brenner MB: Lymphocytes bearing antigen specific $\gamma\delta$ T cell receptors accumulate in human infectious disease lesions. *Nature* 1989;339:544–548.
- 20 Abo T, Sugawara S, Seki S, Fujii M, Rikiishi H, Takeda K, Kumagai K: Induction of human TCR $\gamma\delta$ ¹ and TCR $\gamma\delta$ -CD2⁺CD3⁻ double negative lymphocytes by bacterial stimulation. *Int Immunol* 1990;2:775–785.
- 21 Seki S, Abo T, Sakihara H, Sugiura K, Kanno A, Rikiishi H, Masuda T, Kumagai K: An appropriate in vitro culture condition for the induction of human TCR $\gamma\delta$ ⁺ cells by heat-killed bacteria. *J Immunol Methods* 1991;139:31–40.
- 22 Kabelitz D, Bender A, Schondelmaier S, Schoel B, Kaufmann SHE: A large fraction of human peripheral blood $\gamma\delta$ ⁺ T cells is activated by *M. tuberculosis* but not by its 65-kD heat shock protein. *J Exp Med* 1990;171:667–679.
- 23 Uyemura K, Klotz J, Pirmez C, Ohmen J, Wang X, Ho C, Hoffman WL, Modlin RL: Microanatomic clonality of $\gamma\delta$ T cells in human leishmaniasis lesions. *J Immunol* 1992;148:1205–1211.