Natural killer activity against human K562 tumour cells during \textit{Plasmodium cynomolgi} malarial infection of rhesus monkeys

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1. SUMMARY

This study assessed the natural killer (NK) cell activity profile during \textit{Plasmodium cynomolgi} infection in rhesus monkeys. There was a significant decrease in the NK cell activity in the peripheral blood leukocytes of infected monkeys during the early, ascending phase of infection. However, as the parasite load decreased, NK cell activity returned to normal levels. This could be correlated with the peak increase in lymphocyte counts. This indicated that a decrease in NK cell activity observed at an earlier stage during an active \textit{P. vivax} malarial infection was a temporary phenomenon.

2. INTRODUCTION

Natural killer (NK) cells constitute a naturally occurring defence mechanism which may play an important role in eliminating spontaneously arising tumour cells [1]. There is some evidence which indicates a possible role for NK cells in immunity to parasites. Solomon et al. [2] found evidence for a possible protective role of NK cells in rat malaria. Eugui and Allison [3] reported a correlation between NK cell numbers and susceptibility to the malarial parasite in different strains of mice, however, a direct role for NK cells in malarial immunity has not been established. Indeed, Wood and Clark [4] have suggested that NK cells may be unimportant in malarial immunity. One report [5] on the status of the changes in human malaria does not give a clear picture of the changes in NK cell numbers during the course of the disease. Previously, it was shown that NK cell numbers in peripheral blood decreased during the active phase of human \textit{P. vivax} infection [6]. In the present study, the monkey malaria model was used to gain an understanding of the changes in the NK cell population during different phases, i.e. ascending (AP), descending (DP), and recovery (RP), of the infection.

3. MATERIALS AND METHODS

3.1. Experimental infection of rhesus monkeys with \textit{P. cynomolgi}

Healthy adult rhesus (\textit{Macaca mulatta}) were purchased locally and quarantined for two weeks...
before initiating a *P. cynomolgi* (from National Institute of Communicable Diseases, Delhi) infection by the transfer of $1 \times 10^6$ infected erythrocytes from an infected monkey to a healthy monkey.

3.2. Parasitaemia and leukocyte monitoring

Parasitaemia and the differential count of leukocytes were monitored at different time periods following infection up to 28 days. Parasitaemia was determined from JSB (Jaswant Singh Bhattacharya strain) stained blood smears as described [7]. The number of parasitized erythrocytes in $1 \times 10^3$ RBCs was counted for the calculation of percent parasitaemia. A differential leukocyte count was also made from the same blood smear by counting the different cell populations in at least 200 fields.

3.3. Isolation of peripheral blood leukocytes (PBL)

Peripheral blood leukocytes were isolated by Ficoll Hypaque density gradient centrifugation as described by Boyum [8], washed three times with plain RPMI medium (200 × g, 10 min for each washing) and suspended in complete medium (RPMI 1640 medium containing 10% (v/v) foetal calf serum). Cells were counted in a haemocytometer and finally adjusted to the required cell concentration in complete medium.

3.4. Natural killer cell assay

NK activity was studied in the monkey PBL using a standard chromium release assay [9] with human K562 tumour cells as target. Briefly, the assays were done in round bottomed, microtitre plates in a 15 h $^{51}$Cr release assay. PBL ($10 \times 10^6$ ml$^{-1}$, 100 μl/well) from normal and infected monkeys were incubated with $^{51}$Cr-labelled target K562 cells ($1 \times 10^4$ cells in 100 μl/well) at different effector:target (E:T) ratios for 15 h at 37°C. At the end of the experiment 100 μl of the assay mixtures (TEST) were removed from each well and the $^{51}$Cr released was counted in an LKB Gamma counter. Spontaneous release (SR) of $^{51}$Cr was studied by incubating an equal volume of medium instead of effector PBL cells with the labelled target K562 cells. To estimate the maximum release (MR) of $^{51}$Cr, $1 \times 10^4$ labelled target cells were suspended in 1.0 ml of distilled water, vortexed well and centrifuged at 500 × g for 10 min. Radioactivity in 0.5 ml of the supernate was determined. The percentage specific lysis of target cells was calculated by the following formula:

\[
\text{Percent lysis} = \frac{\text{TEST cpm} - \text{SR cpm}}{\text{MR cpm} - \text{SR cpm}} \times 100
\]

4. RESULTS

Five rhesus monkeys were infected with *P. cynomolgi* and the course of infection in each case was followed by determining the percentage parasitaemia in peripheral blood erythrocytes. Percentage of parasitaemia increased over a period of about sixteen days (the ascending phase) and peaked at an average of 11.5 ± 5.27. Thereafter, over a period of about one week (the descending phase) the parasitaemia declined to an average of 0.08 ± 0.1. This was followed by the recovery phase and during this phase parasites were eliminated from the peripheral blood stream and the animals were microscopically negative for parasites in blood. During each of the phases of infection the levels of anti K562 NK cell activity in the PBL was examined at three E:T ratios (100, 50 and 25:1). The NK cell activity in an uninfected

![Fig. 1. NK activity in peripheral blood derived from normal rhesus monkeys at different stages of *P. cynomolgi* infection. AP, ascending phase; DP, descending phase; RP, recovery phase. Levels of significance were determined by the Student's t-test. * $P < 0.01$, ** $P < 0.05$.](image)
Table 1

*P. cynomolgi* infection and differential leukocyte counts in rhesus monkeys

<table>
<thead>
<tr>
<th>Day of Infection</th>
<th>Parasitaemia</th>
<th>Polymorph (Neutrophil)</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Eosinophil</th>
<th>Basophil</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>0.06±0.04</td>
<td>58±7.64</td>
<td>31±3.05</td>
<td>4±1.5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>0.6±0.1</td>
<td>45±6.0</td>
<td>48±7.5</td>
<td>7±0.6</td>
<td>2±1.5</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>1.03±0.15</td>
<td>50±5.5</td>
<td>45±11.0</td>
<td>7±2.5</td>
<td>1±0.0</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>1.8±0.2</td>
<td>30±7.0</td>
<td>51±3.05</td>
<td>8±1.0</td>
<td>1±0.0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>3.5±1.07</td>
<td>41±3.6</td>
<td>50±2.0</td>
<td>8±5.5</td>
<td>1±0.7</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>11.5±5.27</td>
<td>44±10.45</td>
<td>47±9.0</td>
<td>8±5.5</td>
<td>1±0.7</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>2.89±1.28</td>
<td>22±2.5</td>
<td>63±6.78</td>
<td>16±4.5</td>
<td>0.8±0.45</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>0.52±0.21</td>
<td>18±3.0</td>
<td>72±7.50</td>
<td>6±2.5</td>
<td>1±0.0</td>
<td>0</td>
</tr>
<tr>
<td>22</td>
<td>0.08±0.1</td>
<td>22±3</td>
<td>67±10.0</td>
<td>8±2.0</td>
<td>2±1.5</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>–ve</td>
<td>40±5</td>
<td>58±7.0</td>
<td>3±1.5</td>
<td>0.5±0.5</td>
<td>0</td>
</tr>
<tr>
<td>28</td>
<td>–ve</td>
<td>50±6</td>
<td>50±0.0</td>
<td>3±1.0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>0 (Normal monkeys)</td>
<td>–</td>
<td>72±2.42</td>
<td>20±2.32</td>
<td>4±1.05</td>
<td>2±1.2</td>
<td>0.3±0.04</td>
</tr>
</tbody>
</table>

Rhesus monkeys were inoculated with *P. cynomolgi* (1×10⁶ cells/monkey) on day 0. Prepatent period was about 8 days. Results shown above represent mean±S.D., values of five infected normal monkeys.

monkeys was also determined in each experiment as a control. The combined results of these experiments, shown in Fig. 1, indicate that there was a significant fall in peripheral blood NK cell activity during the ascending phase (AP) of the infection. The descending phase (DP) of the infection was associated with normal or slightly elevated NK cell activity (Fig. 1) whereas the levels of NK cell activity in control monkeys and monkeys in the post infection, recovery phase (RP) were not significantly different.

The differential leukocyte counts in monkeys infected with *P. cynomolgi* showed that with the increase in parasitaemia, to day 16 there was a varying neutrophil count to day 16 accompanied by an increasing lymphocyte count to day 20 (Table 1). The lymphocyte peak value (72±7.5%) was observed in the declining phase of the infection. It is interesting to note that the increase in NK activity during this period correlated with the increase in lymphocyte counts. As with lymphocytes, the monocyte count increased to day 16–18 of the infection. The eosinophil and basophil counts remained low.

5. DISCUSSION

Previously, a decline in the count of peripheral blood NK cells was reported in patients with *P. vivax* malaria [6]. A follow-up study was difficult since the patients were not available for regular blood sampling. Moreover, the patients were on chemotherapy which could interfere with the pattern of changes in NK cell numbers during the course of infection. Due to its similarity with human *P. vivax* infection [10], the *P. cynomolgi* infection model of monkeys was used to assess the changes in NK cell activity during the course of infection. The results indicated a significant decline in peripheral blood NK cell activity during the ascending phase (AP) of the infection as observed previously in *P. vivax* malarial patients [6]. However a decline in blood NK cells does not necessarily indicate suppression of the NK cell population. In tumour-bearing mice relocation of NK cells to the tumour mass may result in an apparent decrease in systemic NK cell numbers [11]. Interestingly, parasitized erythrocytes can competitively inhibit the lysis of K562 cells by the NK cells [12]. This finding may indicate that the infected erythrocytes may interact with the NK cells. If such an interaction can indeed take place, it is possible that the NK cells may relocate to liver and spleen containing infected cells thus resulting in an apparent decline in circulating NK cells. The increase in NK cell activity during the descending (DP) and recovery phases (RP) could result from the migration of NK cells back into the blood. In this context it should be noted that
the NK cells are capable of lysing several target cells and do not die after a single encounter with the target [13]. These suggestions about the migration of NK cells however are purely hypothetical at present and would require further work for validation. The increase in NK activity during the declining phase of infection seems to correlate with the maximum increase in the lymphocyte count. Interferon mediated NK cell activation was reported in malarial patients [14]; this could contribute to the normalization of NK cell numbers during the later phases of infection.

The differential leukocyte cell counts obtained in this study were reasonably similar to earlier observations in monkeys infected with \textit{P. brasilianum}, \textit{P. coatneyi} and \textit{P. falciparum}, as shown by the increase in blood lymphocyte and monocyte count in infected monkeys [15]. Interestingly, the neutrophil count rose during the infection and fell between days 18 and 22, but tended to go up again (days 25–28) in a secondary surge. The relevance of these changes is not understood; the data on changes in the counts of other types of leukocytes may indicate an important role for these cells in immunity to the malarial parasite. There is a need for additional work in this area.

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REFERENCES


