T AND B LYMPHOCYTES AND THEIR SUBPOPULATIONS IN PERIPHERAL BLOOD IN RABBITS EXPERIMENTALLY INFECTED WITH Fr-2 STRAIN OF VIRAL HAEMORRHAGIC DISEASE (VHD) VIRUS

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Abstract

Kinetic alterations in the number of T and B lymphocytes and their subpopulations (Th, Tc/Ts, activated T and B lymphocytes) in peripheral blood of rabbits were examined following experimental infection with VHD virus, French strain, Fr-2. The virus was found to increase the number of the blood cells. The increase was most pronounced and most protracted in the number of Th, T and Tc/Ts lymphocytes.

Key words: rabbit, VHD virus, lymphocytes, lymphocyte subpopulations.

Traditionally, studies on immunological phenomena associated with VHD virus infection focus mainly on cellular and humoral non-specific immunity (9, 10, 16, 18 - 20), but to a lesser degree on a specific immunity (2, 5, 9 - 16, 19, 20). Additional studies usually concentrated on experiments involving quantitative determinations of T and B lymphocytes (2 - 15, 20) as well as serum immunoglobulins of class G only (10, 19, 20). Furthermore, Chinese authors who employed experimentally infected rabbits in their experiments observed increased values of T and B lymphocytes in a rosette test (2, 15, 16, 18). In our preliminary experiments, where determinations T and B lymphocytes in flow cytometry were done, there were changes in dynamics of the mentioned cells, which depended on the strain of the virus used and its dose (3 - 14, 20).

The studies aimed at recording kinetic alterations in the number of T, Th, Tc/Ts, B and activated T and B lymphocytes in peripheral blood of rabbits experimentally infected with 100% lethal dose of VHD virus, HI – 1024, French strain, Fr-2. The virus originated from rabbits naturally infected and deceased in France and had the form of a 20% liver homogenate, purified by chloroform treatment and centrifugation (19). A control group consisted of 10 animals, which in the same manner were given sterile distilled water. In all the rabbits blood for tests was sampled from the marginal ear vein just before infection (hour 0) and then at hours 4, 8, 12, 24, 36, 48, 52, 56 and 60 following the infection. No examinations were carried out at further time points since the nine still surviving rabbits died before the 72 h of the experiment.

Content (%) of T and B lymphocytes and of their subpopulations was estimated in a flow cytometer (Cytron Absolute, Ortho) employing monoclonal antibodies (Serotec), detecting the following surface CD structures: CD5 (T lymphocytes ), CD4 (Th lymphocytes), CD8 (Tc/Ts lymphocytes), CD19 (B lymphocytes) and CD25 (activated T and B lymphocytes). The presence of specific VHD virus antibodies was also examined in sera of the infected rabbits.

Material and Methods

The studies were performed on 40 mixed breed rabbits, each 3.0 kg to 3.8 kg body weight, in national norms representing animals of the group CV-III, i.e. originating from a breeding colony under a full veterinary supervision (1). Thirty rabbits were intramuscularly infected with 100% lethal dose of VHD virus, HI – 1024, French strain, Fr-2. The virus originated from rabbits naturally infected and deceased in France and had the form of a 20% liver homogenate, purified by chloroform treatment and centrifugation (19). A control group consisted of 10 animals, which in the same manner were given sterile distilled water. In all the rabbits blood for tests was sampled from the marginal ear vein just before infection (hour 0) and then at hours 4, 8, 12, 24, 36, 48, 52, 56 and 60 following the infection. No examinations were carried out at further time points since the nine still surviving rabbits died before the 72 h of the experiment.

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Results of the studies were statistically analysed using Student’s t-test, comparing at P=0.05 the values obtained in infected and control animals.
Results

Results of the studies, in the form of arithmetic means, are presented in Table 1 and, in a graph form, on Figs 1 and 2. Significant differences were appropriately marked.

The analysis of the obtained results indicated the increase in the number of T and B lymphocytes and their subpopulations in rabbits experimentally infected with VHD virus (Table 1). The increase was most persistent and most pronounced in the number of Th lymphocytes (hours 12, 24, 36, 52, 56, 60), followed by T lymphocytes (hours 12, 36, 52, 56, 60), Tc/Ts lymphocytes (hours 8, 12, 36, 56), but it was much less pronounced and less persistent in the number of activated T and B lymphocytes (hours 24, 36, 56) and B lymphocytes (hours 24, 52, 56). Analysis of alterations involving variability in the number of the tested lymphocyte subpopulations (Figs 1 and 2), as compared to their number before infection at hour 0, demonstrated that at any time points examined the alterations in lymphocyte subpopulations were involved principally in increased and very rarely in decreased number of the cells. The increasing tendency started already at hour 8 for T lymphocytes, at hour 12 for Th lymphocytes, at hour 36 for Tc/Ts lymphocytes and lymphocytes with CD25 marker, and at hour 48 for B lymphocytes. The alterations persisted till the end of the observation, except for hour 48 for T, Th, and Tc/Ts lymphocytes, and activated T and B lymphocytes. VHD virus antibodies were not detected in the ELISA.

Discussion

Comparing the obtained results to those related to kinetic alterations in the number of lymphocyte populations and subpopulations (T, Th, Tc/Ts, B, and activated T and B lymphocytes) in rabbits experimentally infected with VHD virus, Polish strain Kr-1, and Czech strain V-351 (3 - 14, 17, 20), one can note that the results are similar and convergent but, nevertheless, they differ depending upon the employed strain of VHD virus and infecting dose of the virus.

Upon comparison of the results obtained in rabbits receiving 100% lethal dose of VHD virus, French strain, Fr-2, with the results obtained in rabbits which received the same dose of VHD virus, Polish strain, Kr-1, (4 - 14, 20), the latter strain increased the number of lymphocytes and their subpopulations of a shorter duration than those induced by Fr-2 but also decreased their number.

Table 1

Percentages of lymphocytes in peripheral blood of rabbits experimentally infected with VHD virus, French strain (Fr-2)

<table>
<thead>
<tr>
<th>Hours after infection</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>24</th>
<th>36</th>
<th>48</th>
<th>52</th>
<th>56</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups and number of animals</td>
<td>Z</td>
<td>K</td>
<td>Z</td>
<td>K</td>
<td>Z</td>
<td>K</td>
<td>Z</td>
<td>K</td>
<td>Z</td>
<td>K</td>
</tr>
<tr>
<td>Lymphocytes T (CD5)</td>
<td>53.4</td>
<td>48.8</td>
<td>51.2</td>
<td>50.3</td>
<td>53.2</td>
<td>49.0</td>
<td>58.5*</td>
<td>49.0</td>
<td>53.4</td>
<td>49.0</td>
</tr>
<tr>
<td>Lymphocytes Th (CD4)</td>
<td>39.7</td>
<td>38.4</td>
<td>39.8</td>
<td>39.0</td>
<td>37.5</td>
<td>38.4</td>
<td>46.5*</td>
<td>38.8</td>
<td>45.1*</td>
<td>40.5</td>
</tr>
<tr>
<td>Lymphocytes Tc/Ts (CD8)</td>
<td>20.6</td>
<td>14.8</td>
<td>19.4</td>
<td>16.1</td>
<td>19.6*</td>
<td>10.5</td>
<td>17.5*</td>
<td>9.6</td>
<td>15.1</td>
<td>15.6</td>
</tr>
<tr>
<td>Lymphocytes B (CD19)</td>
<td>15.9</td>
<td>16.6</td>
<td>15.2</td>
<td>13.4</td>
<td>12.0</td>
<td>9.2</td>
<td>11.2</td>
<td>9.5</td>
<td>14.9*</td>
<td>10.6</td>
</tr>
</tbody>
</table>

Z – infected animals; K – control animals; A - animals deceased before the 72th h of the experiment *- difference significant at P=0.05
Infection with Kr-1 was followed by increased and decreased number of T, B, Th and Tc/Ts lymphocytes, and an increase in activated T and B lymphocytes only. However, it should be added that the detected decrease in T, B, and Th lymphocytes following infection with Kr-1 strain was noted only at individual time points after the infection and in the case of Tc/Ts lymphocytes the decrease in their number developed 24-48 h before death of the rabbits. Comparing the results obtained using 100% lethal dose of Fr-2 strain, with those recorded in rabbits infected with 25%, 50% or 75% lethal dose of the strain (10, 11, 12, 14, 20), the latter three doses were found to induce slightly shorter elevations of the lymphocytes and their subpopulations. Moreover, infection with the 25% lethal dose was observed to be associated, in addition, with a very short decrease in the number of Tc/Ts and T lymphocytes. On the other hand, the results obtained following the infection with 25%, 50% or 75% lethal dose of Fr-2 strain, to those recorded in rabbits receiving the same doses of the Polish strain, Kr-1 (6, 8, 10, 14, 15, 20), the latter strain was found to induce slightly more protracted alterations, particularly when 50% lethal dose was used, and it was associated more frequently with a decrease than with an increase in the number of the studied lymphocytes and their subpopulations. Following infection with Kr-1 strain, a decrease in the number of the cells was recorded not only following the 50% lethal dose (when the principal decrease in cell levels has involved CD4+ and CD5+ cells) but also following infection with 25% and 75% lethal doses of the strain. Following infection with 25% lethal dose of Kr-1 strain, the decrease involved CD4+ and CD25+ lymphocytes and following infection with 75% lethal dose of the strain it involved D4+, CD8+ and CD19+ lymphocytes.

It should be added that preliminary results on kinetic alterations in the number of lymphocytes and their subpopulations in peripheral blood of rabbits infected with 100% lethal dose of VHD virus, Czech strain V-351 (3, 4) demonstrated that the antigen induced more frequently a decrease than increase in the cell number. The most pronounced decrease included mainly CD4+, CD5+ and CD19+ lymphocytes while the analogous increase pertained to CD25+ cells. It should be added that the increased numbers of the cells at various time and to a variable extent, pointed to stimulatory as well as inhibitory effects of the virus on the rabbit immune system. The elevated number of Tc/Ts lymphocytes during the infection seem to reflect inhibitory effects of VHD virus on immune response in the infected rabbits. This was indirectly confirmed by the results on alterations in the number of B lymphocytes and activated T and B lymphocytes (Figs 1 and 2). Results obtained in rabbits infected with 100% lethal dose of the French strain (Fr-2) of VHD virus point to a stimulatory action of the antigen on the studied lymphocytes and on their subpopulations, in particular on Th, T and Tc/Ts lymphocytes. The distinct kinetics of the alterations noted following infection with the Polish strain, Kr-1, and the Czech strain, V-351, provides evidence for a distinct immunogenic potential of the VHD virus strains, originating from different biotopes and documents the unproven till now role of specific cell-mediated immunity in the acutely progressing disease. The data corroborate the earlier results of our own (9, 10, 19, 20), in which the variable immune reactivity was demonstrated for the five Polish strains (KGM, SGM, MAL, BLA, Kr-1), two French strains (Fr-1, Fr-2) and one Czech strain (V-351) of VHD virus, in their capacity to induce non-specific immunity and the appearance of serum antibodies.

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