SERUM PROTEIN PROFILE OF MICE DURING INFECTION WITH SINGLE AND REPEATED DOSES OF HYMENOLEPIS NANA EGGS

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Abstract. Significant alterations in serum protein of mice following Hymenolepis nana infection were observed. These changes were recorded as decrease in albumin, increase in gamma globulin and a temporary rise in alpha-1, alpha-2 and beta globulins. The decrease in albumin and increase in gamma globulin occurred as early as on 1st day after infection. The alpha-1 and alpha-2 globulins did not show definite profile during infection. The beta globulin predominantly increased till the day 20 post infection and thereafter generally decreased. Repeated infection did not enhance any further alterations in serum protein. There was no significant correlation between infection dose levels and serum protein changes.

It is reported that animals infected with helminth parasites show marked changes in their serum protein fractions in predictable patterns (Stauber 1954, Wall 1958). In cestode infection, scanty information is available on the serum protein changes due to Hymenolepis nana infection in rodents. Noda (1956) and Katiyar et al. (1973) have reported decrease in albumin and increase in gamma globulin fraction in mice and rats respectively due to H. nana infection.

The present communication provides an objective effort to determine alterations in serum protein of mice infected with single and repeated doses of H. nana eggs.

MATERIAL AND METHODS

Adult Swiss albino male mice (Mus musculus albinus) weighing 20–25 g raised in H. nana free environment were used as the hosts. The infection was given orally following the method of Heyneman (1962). Five groups consisting of 30 mice each were used. Three groups were infected with a single dose (group 1 — 1000, group 2 — 2000, group 3 — 4000 eggs), fourth group with repeated doses (group 4 — 1000 + 2000 + 4000 eggs at 7-day intervals) and the fifth group was kept as uninfected control. Three mice from each singly infected and control group were killed on days 1, 5, 10, 15, 20, 25, 30, 40, 50 and 60 and 3 mice from repeatedly infected group were also killed after receiving final infection dose. Blood was collected aseptically by cardiac puncture on the same designated days for serum separation. Serum protein analysis was carried out on low voltage horizontal (Systronic — Type 601) electrophoresis apparatus following the method of Bhopale and Johri (1976). The strips were stained with modified bromophenol blue solution and differentiated in 0.5 % glacial acetic acid. The data obtained from the experimental groups were submitted to statistical analysis (Student’s t-test and coefficient of correlation r'). A confidence limit was established at the 5 % level of significance as per the formula given by Armitage (1971) in order to ascertain if there was any significant variation on a particular day in serum protein of experimentally infected animals as compared to those from uninfected control. To find out whether further alterations in the serum protein had taken place following repeated infections, data obtained from group 4 were compared with those of group 3 which had received a single dose infection of 4000 eggs.

RESULTS

The results presented graphically in Fig. 1 indicate a decrease in albumin, increase in gamma globulin and a temporary increase in alpha and beta globulins following single or repeated doses of H. nana eggs.
Fig. 1. Graphic pattern of serum protein components of various groups following *H. nana* egg infection. Mean values falling within the confidence limit were considered as insignificant whereas outside the limit as significant at 5%.
The albumin concentration remained significantly low in all the infected groups during the entire period of observation (Fig. 1). There was a steep fall in its concentration as early as on the day 1 reaching to maximum depletion on the day 20 post infection in group 1 (24.83 %), group 2 (21.16 %) and group 3 (14.72 %) and on day 15 post challenge infection in group 4 (25.18 %) when compared with control (46.97 %) uninfected group. Thereafter, it increased but did not return to its normal level in any groups.

The alpha fractions (alpha-1 and alpha-2) did not show any definite pattern in any group. The alpha protein level in the infected groups were mostly above the controls and in 50 % of the cases the difference was statistically significant.

An initial increase in the beta globulin following infection was evident from day 5 in group 2 (32.10 %), day 10 in group 1 (32.41 %) and group 3 (32.06 %) as compared to control (26.77 %). Thereafter, it showed a progressive increase up to day 20 when it reached its maximum concentration in group 1 (33.03 %), group 2 (33.8 %) and group 3 (35.65 %). On repeated infection in group 4, it exhibited a slight fluctuation remaining more or less near the normal level although it increased significantly on days 10 (28.85 %), 20 (28.94 %), 25 (28 %) and 50 (28.98 %) post challenge infection (Fig. 1).

The most remarkable change occurring as a result of infection was a sudden and marked increase in the gamma globulin fraction, beginning with the day 1 after infection and remained high till the end of experiment (Fig. 1). It showed a continuous increase reaching the maximum level after infection on days 15 in group 2 (19.41 %, i.e. 2 1/2 fold increase), 20 in group 1 (18.14 %, i.e. 2 fold increase) and 30 in group 3 (25.17 %, i.e. 3 fold increase) as compared to control (7.14 %). In repeatedly infected group, it showed two peaks, i.e., on day 15 (21.66 %) and 50 (20.28 %) post challenge infection which could be attributed to the fact that mice in this group had been repeatedly infected before serum samples were taken.

The total globulins increased in their concentrations and showed a reverse pattern to that of albumin concentration. The albumin globulin ratio remained below its normal level for the entire period of observation due to decrease in albumin and increase in total globulins in all the groups.

On statistical analysis of the data summarized in Table 1, it was seen that there was no significant correlation between infection dose levels and serum protein changes (P > 0.050). This indicates that serum protein alterations are not in accordance with the infection dose level.

DISCUSSION

It is evident from our studies that serum protein alterations were not in accordance with the increasing dose levels in singly infected groups (P > 0.050). Statistical analysis of the data further revealed that repeated doses of infection did not induce further alterations in the serum protein. In repeatedly infected group the changes in serum protein had already taken place due to previous infections prior to the challenge infection. The possible explanation as to why repeated infections did not induce further alterations could be given as when the host system has once reached the immune state, it expelled out subsequent doses of infection, thereby preventing further onset of infection which otherwise could have induced further alterations in the serum protein. These results are in agreement with Krant (1956), Aljeboori and Ivey (1970) and Mishra (1972) who did not find correlation between the infection level and the extent of changes in serum protein during infection of larvalcestode *Cysticercus faciolaris* in rats, *Toxocara canis* in baboons and *Angiostrongy-
Table 1. Average value of serum protein components of mice infected at various infection dose levels. (Values expressed are pooled average ± standard error for 60 days period of observation from each group)

<table>
<thead>
<tr>
<th>Group</th>
<th>Albumin</th>
<th>Globulins</th>
<th>Total globulins</th>
<th>A/G Ratio (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean concentration of fractions (percent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alpha-1</td>
<td>Alpha-2</td>
<td>Beta</td>
</tr>
<tr>
<td>Group 1*</td>
<td>35.54</td>
<td>6.43</td>
<td>16.22</td>
<td>29.07</td>
</tr>
<tr>
<td>(1000 eggs)</td>
<td>± 1.73</td>
<td>± 0.29</td>
<td>± 0.73</td>
<td>± 0.79</td>
</tr>
<tr>
<td>Group 2*</td>
<td>31.37</td>
<td>7.81</td>
<td>16.81</td>
<td>29.59</td>
</tr>
<tr>
<td>(2000 eggs)</td>
<td>± 1.86</td>
<td>± 0.48</td>
<td>± 0.48</td>
<td>± 0.77</td>
</tr>
<tr>
<td>Group 3*</td>
<td>28.09</td>
<td>7.03</td>
<td>17.26</td>
<td>29.57</td>
</tr>
<tr>
<td>(4000 eggs)</td>
<td>± 2.71</td>
<td>± 0.63</td>
<td>± 1.49</td>
<td>± 0.97</td>
</tr>
<tr>
<td>Group 4*</td>
<td>29.89</td>
<td>7.81</td>
<td>17.44</td>
<td>27.87</td>
</tr>
<tr>
<td>(1000 + 2000</td>
<td>± 1.08</td>
<td>± 0.36</td>
<td>± 0.77</td>
<td>± 0.28</td>
</tr>
<tr>
<td>± 4000 eggs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 5</td>
<td>46.97</td>
<td>5.33</td>
<td>13.83</td>
<td>26.77</td>
</tr>
<tr>
<td>(control</td>
<td>± 0.75</td>
<td>± 0.22</td>
<td>± 0.25</td>
<td>± 0.41</td>
</tr>
<tr>
<td>uninfected)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation</td>
<td>P &gt; 0.050</td>
<td>NS</td>
<td>P &gt; 0.050</td>
<td>NS</td>
</tr>
<tr>
<td>coefficient</td>
<td>NS</td>
<td>NS</td>
<td>P &gt; 0.050</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS indicates nonsignificance

* Serum protein fractions values of group 1, 2, 3 and 4 are statistically significant (P < 0.050) from the control group 5, except where indicated as not significant NS (P > 0.050)
gylus cantonensis in rats, respectively. Similarly Bhopale and Johri (1975) have also shown that repeated infections of Ancylostoma caninum in mice did not bring further significant alteration in the serum protein as compared to the single-dose infection.

The present findings reveal that H. nana infection in mice induces significant alterations in serum protein as evident by decrease in albumin, increase in gamma globulin and a temporary rise in alpha and beta globulins resulting in an increase of globulins and decrease of A/G ratio. Such changes in serum protein have also been reported in other intestinal helminths as well, for example, Haemonchus contortus in sheep (Kuttler and Marble 1960), trichuriasis in pigs (Medzyavichyus et al. 1968), ascariasis in Egyptian children (Wishany et al. 1969), Trichostrongylus colubriformis and Haemonchus contortus mixed infection in sheep (Zajíček et al. 1972). These variations in serum protein reflect gross physiological alterations within the host system due to pathogenesis and host’s immune mechanism. In the present experiment, the albumin depletion during an initial phase of infection and increase in alpha globulins could be due to severe damage of intestinal villi produced by the oncospheres and subsequent developmental stages of worm. Kramar et al. (1974) demonstrated excretion of albumin in H. nana infection in rats as early as within 1 min. after infection. In our study, albumin depletion was maximum during active reproductive phase of worm while worm’s nutritional requirement of albumin was increased (Katiyar et al. 1973). Increase in beta and gamma globulin in the present study are attributed to an immunological response induced by the developmental phases of the worm. The maximum increase in these two components was seen during maturity of worm. In H. nana infection, gamma globulin is known to contain antibodies (Noda 1956) which have a protective action (Diconza 1969). Thus a constant high level of gamma globulin is responsible to a certain extent for the expulsion of worms and subsequent infections, representing an immune state of the host.

ИЗМЕНЕНИЯ БЕЛКА СЫВОРОТКИ У МЫШИ ПОСЛЕ ОДНОКРАТНОГО И ПОВТОРНОГО ЗАРАЖЕНИЯ ЯЙЦАМИ ЦЕСТОДЫ HYMENOLEPIS NANA

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Резюме. После заражения мыши цестодой Hymenolepis nana обнаружены значительные изменения белка сыворотки, а именно понижение количества альбумина, повышение количества гамма-глобулина и временное повышение альфа-1, альфа-2 и бета глобулинов. Понижение количества альбумина и увеличение гамма-глобулина произошло уже на 1-й день после заражения. Количество альфа-1 и альфа-2 глобулины менялось во время инфекции. Количество бета-глобулина повышалось до 20-го дня после заражения и потом обыкновенно понижалось. Повторительное заражение не вызывало других изменений. Не наблюдалось отношение между уровнем доз заражения и изменениями белка сыворотки.

REFERENCES


BHOPALE M. K., JOHRI G. N., Serum protein pattern of mice during infection with single


HEYNEMAN D., Studies of helmint immunity.


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