

ANCYLOSTOMA CANINUM: A REPORT ON THE PERIPHERAL EOSINOPHILIA IN NAIVE AND IMMUNIZED SWISS ALBINO MICE

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Abstract. The number of peripheral eosinophils was counted in female Swiss albino mice on 1, 4, 9, 16 and 30 days after infection with various single (500, 1 000 and 2 000) and weekly repeated (500 + 500 + 1 000, 1 000 + 1 000 and 1 000 + 1 000 + 2 000) doses of filariform *Ancylostoma caninum* larvae. The eosinophil response was significantly higher in infected than in uninfected mice and reached a peak on day 16 in naive and day 4 and 9 in immunized mice. Immunized mice were significantly more eosinophilic than the naive mice. An attempt has been made to correlate eosinophilia with immunity of mice to *A. caninum* larvae.

Peripheral blood and tissue hypereosinophilia are characteristics of diverse parasitic infestations in man and animals. Many workers have reported an increase in the number of eosinophil leucocytes in blood during experimental infection of various helminths, e.g. *Haemonchus contortus* (Soulsby 1960), *Trichinella spiralis* (Zaiman et al. 1962, Basten et al. 1970, Basten and Beeson 1970, Ismail and Tanner 1972), *Toxocara canis* (Sharp and Olson 1962, Olson and Schulz 1963), *Trichostrongylus colubrifomis* (Rothwell and Dineen 1972), *Ascaris suum* (Nielsen et al. 1974), *Schistosoma* spp. (Colley 1974, Sturrock et al. 1977), *Taenia taeniaeformis* (Ansari and Williams 1976, Ansari et al. 1976) and *Nippostrongylus brasiliensis* (Ogilvie et al. 1978). Recently Rothwell and Dineen (1972) and David (1977) have implicated an important role for the eosinophils in worm expulsion and/or destruction.

Since *Ancylostoma caninum* undergoes extensive migration and distribution in mice after oral infection (Bhopale and Johri 1975, 1978a) leading to the alterations in serum protein levels (Bhopale and Johri 1978b, 1979), intestinal mast cell numbers (Vardhani and Johri 1979a) and histamine content (Vardhani and Johri 1979b) without developing into adults, this model serves well for the study of mechanism of immunity as there are no undue complications caused by antigenic variations during development. The present investigation records the changes in the number of eosinophilic leucocytes in peripheral blood of Swiss albino mice after infection with various single and repeated doses of *A. caninum* larvae and their relationship to the immune response.

MATERIAL AND METHODS

Female Swiss albino mice having body weight of 18-20 g were used in these experiments. They have been originally obtained from Haffkine Pharmaceutical Corporation Ltd., Bombay and have been closely bred among themselves for the last 10 years in this laboratory. They were provided with Gold Mohur Mice feed by Hind Lever Co., Bombay and clean drinking water ad libitum. The animal house was maintained under ideal conditions of temperature, light and ventilation. The light-darkness cycle was 12 : 12 hours. Filariform *A. caninum* larvae were obtained from cultures of the faeces of an experimentally infected dog maintained in the laboratory (Sen et al. 1965) and administered orally to the mice in required doses. Blood was collected between 2 to 4 p.m. each time to minimize the diurnal variation (Basten et al. 1970). Prior to bleeding, the mice were warmed under

a 100 watt lamp and a tail vein was punctured with an aseptic sharp blade. The first few drops of blood were discarded and subsequent drops were used to prepare two smears from each mouse. Differential counts of Giemsa stained slides were made of 200 leucocytes per mouse.

Single doses of 500, 1 000 and 2 000 larvae were administered to each mouse of groups A, B and C respectively. Repeated infections of 500 + 500 + 1 000, 1 000 + 1 000 and 1 000 + 1 000 + 2 000 larvae at an interval of 7 days were given to each mouse of groups D, E and F respectively. All the six experimental groups consisted of five mice each and four uninfected mice constituted a control group. The blood smears were examined on 1, 4, 9, 16 and 30 days after infection in singly infected groups and after the last infection in repeatedly infected groups. The eosinophil counts in six experimental groups were compared by means of t-tests with those in uninfected group.

RESULTS

The results are presented in Figs. 1 and 2. Significantly higher eosinophil counts occurred in all the experimental groups in comparison to uninfected group ($P < 0.001$, $t = 5.41$ with seven degrees of freedom) throughout the experiment. An exception was group C mice infected with 2 000 larvae each in which eosinophil counts did not differ from that of controls at day 1.

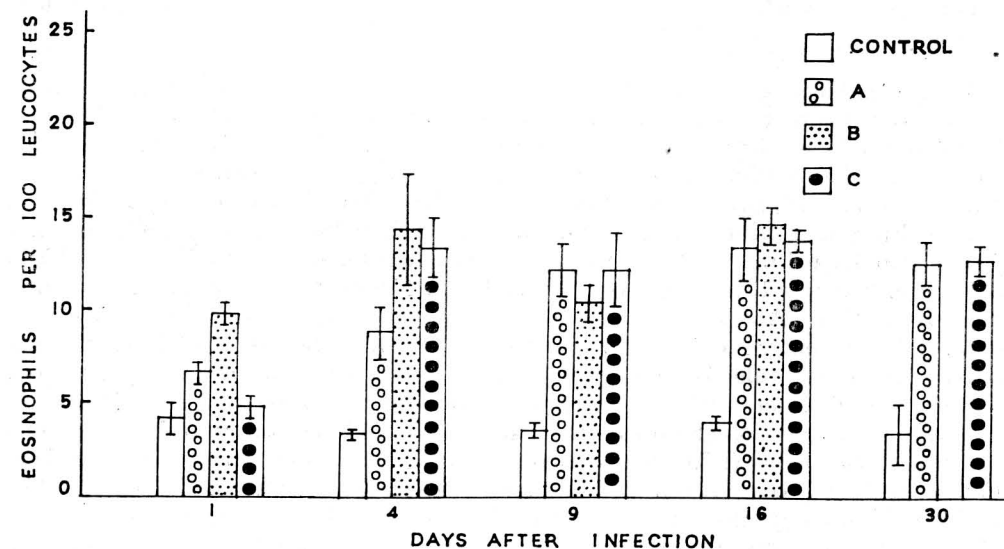


Fig. 1. Mean number of eosinophils ($\pm$ standard deviation) per hundred leucocytes in mice infected per os with single doses of 500 (A), 1 000 (B) and 2 000 (C) infective *Ancylostoma caninum* larvae.

In all the singly infected groups A, B and C, the maximum number of eosinophils was recorded at days 16. In case of repeatedly infected groups, it was at day 4 in groups D and E, and at day 9 in F. The eosinophilia in all the experimental groups was evident even one month after infection. Observations in group B were not possible on day 30 because of the mortality of animals in this group.

DISCUSSION

It is of common knowledge that tissue invading parasites are more eosinophilogenic than the lumen dwelling ones and this applies well to *A. caninum* in mouse as the present investigation clearly shows. Different single or repeated infections of *A. caninum*

larvae in mice evoked a significant peripheral eosinophilia which was consistent throughout the experiments. These results are in agreement with the observations by Soulsby (1960), Sharp and Olson (1962), Zaiman et al. (1962), Olson and Schulz (1963), Basten et al. (1970), Basten and Beeson (1970), Ismail and Tanner (1972), Colley (1974), Nielsen et al. (1974), Ansari and Williams (1976), Sturrock et al. (1977) and Ogilvie et al. (1978) who reported marked eosinophilia after experimental infections with various helminths.

The onset of eosinophilia occurred on the very first day after infection in all the experimental groups except C, and thus coincided with the migration of larvae to the muscles (Bhopale and Johri 1975, 1978a). Among the singly infected or naive mice a pronounced increase in eosinophil numbers was found in group B while in A and C it was less marked. This can be explained on the basis of immune response of mice to lower and higher doses of infection as described by Bhopale and Johri (1975, 1978a) who found that a dose of 500 larvae was unable to stimulate a measurable immune response and the 2 000 larval dose was so high as to result in a rapid

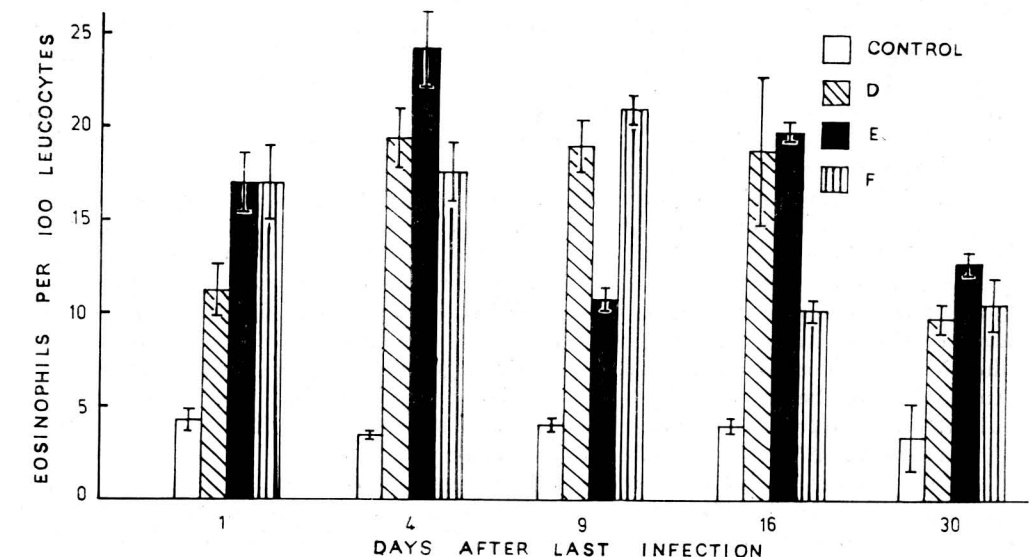


Fig. 2. Mean number of eosinophils ($\pm$ standard deviation) per hundred leucocytes in mice infected per os with weekly repeated doses of 500 + 500 + 1 000 (D), 1 000 + 1 000 (E) and 1 000 + 1 000 + 2 000 (F) infective *Ancylostoma caninum* larvae.

and heavy expulsion of larvae. In all the singly infected groups, the maximum number of eosinophils was found at 16 days when all the recovered larvae get distributed either in brain or muscles of various regions of the body, thereafter the number of eosinophils began to decline up to 30 day with decreasing larval recoveries (Bhopale and Johri 1975). A maximum increase in serum globulins of mice infected with 1 000 larvae on day 9 and of mice infected with 500 and 2 000 larvae on day 15 (Bhopale and Johri 1978b) suggests that an interaction of antibody and antigen might have attracted a large number of eosinophils resulting in a peak eosinophil response in all the singly infected groups (A, B and C) on day 16. Nielsen et al. (1974) also associated peripheral eosinophilia of normal mice with immunity to migrating *A. suum* larvae and positive indirect haemagglutinin antibody titers. As the larvae are

retained in the muscles up to one year after infection without any granuloma formation (Nichols 1956), their presence in this abnormal host may result in a persistent eosinophilia as observed on day 30, which does not show any sign of dropping to normal values. Our results differ from that of Olson and Schulz (1963) who noted a dose-dependent relationship, where highest mean numbers of eosinophils were associated with heaviest infections, and of Ismail and Tanner (1972) who also observed a positive correlation of peak eosinophil numbers with the inoculating dose and with the number of muscle larvae.

Eosinophilia was much more remarkable in repeatedly infected or immunized groups both in terms of earlier onset and higher values than in naive mice; counts on day 1 in groups D, E and F being comparable/higher than the maximum counts at days 16 in groups A, B and C. Similar results have been obtained by Dineen and Rothwell (1972), Nielsen et al. (1974), Ansari et al. (1976) and Ogilvie et al. (1978) who also observed that eosinophil response reaches its peak earlier in immunized than in unimmunized animals. The peak eosinophil numbers in repeatedly infected groups on days 4 and 9 correlate well with earlier and greater expulsion of larvae from immunized mice (Bhopale and Johri 1978a). Among the repeatedly infected groups, mice of E (1 000 + 1 000 larvae) showed a generally higher eosinophilic response than of D (500 + 500 + 1 000 larvae) which were infected with the same total number of larvae, though in three parts in contrast to two in E, thus a dose of 1 000 larvae elicited a good eosinophil response (as seen in group B) which was evident after challenge with the same dose.

Though incidence of secondary immune response in mice infected repeatedly with *A. caninum* larvae was proved by greater and earlier loss of larval burden in comparison to that from singly infected or unimmunized mice by Bhopale and Johri (1978a), these authors did not observe a significant correlation between increase in globulins and secondary immune response (Bhopale and Johri 1979). They, however, asserted that as intestinal tract plays a major role in expulsion of the larvae, a static globulin level does not mean an absence of antigen-antibody reaction in this system. An interaction between antigen and antibody has been found to secrete various substances including an eosinophilchemotactic substance which attracts eosinophils (Ansari et al. 1976); this explains the earlier onset of eosinophilia in the immunized mice. The present authors in the light of findings by Rothwell and Dineen (1972) and David (1977), therefore, speculate an important role for eosinophils in immunity of Swiss albino mice to *A. caninum* larvae. Further studies on the role of eosinophils in expulsion and/or destruction of *A. caninum* larvae are in progress.

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ANCYLOSTOMA CANINUM: ПЕРИФЕРИЧЕСКАЯ ЭОЗИНОФИЛИЯ У НОРМАЛЬНЫХ И ИММУНИЗИРОВАННЫХ БЕЛЫХ МЫШЕЙ Н. Коле и Г. Н. Джори

Резюме. Проводили учет периферических эозинофилов у самок белых мышей на 1-ый, 4-ый, 9-ый, 16-ый и 30-ый день после заражения разными, вводимыми одновременно (500, 1 000, 2 000) и еженедельно (500 + 500 + 1 000, 1 000 + 1 000, 1 000 + 1 000 + 2 000) дозами личинок *Ancylostoma caninum*. Эозинофилия была значительно выше у зараженных, чем у незараженных мышей и достигала пика на 16-ый день у нормальных и на 4-ый и 9-ый день у иммунизированных мышей. Иммунизированные мыши оказывали гораздо высшую эозинофилию, чем нормальные мыши. Сделана попытка установить корреляцию между эозинофилией и иммунитетом мыши к личинкам *A. caninum*.

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