The influence of a *Hymenolepis diminuta* infection on peripheral blood leukocytes in mice

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Summary

Infection of mice with *H. diminuta* has an effect on the percentage of neutrophils and eosinophils in peripheral blood. The increase of the number of neutrophils is limited to the period around day 7 after primary infection. The peaks in the eosinophil response occur on day 15 after primary infection and on day 3 after reinfection. This shows a relation between the cellular response in blood and the rejection of *H. diminuta* by the mouse. Under our experimental conditions, the rejection process lasts from day 7 up to day 14 after primary infection and from day 0 up to day 9 after reinfection. The above-mentioned effects on the leukocytes in the blood offer additional indications for the role of neutrophils and eosinophils in the immunological reaction to intestinal parasites. In many parasitological models the cellular response in peripheral blood can be used as measure for the (local) reaction to the parasite, but in the present model of *H. diminuta* the cellular response in peripheral blood is different and less clear than the reaction described in the lamina propria of the jejunum.

Key Words: *Hymenolepis diminuta*, leukocytes, blood.

Resumen

La infección de ratones con *Hymenolepis diminuta* induce variaciones en la proporción de neutrofilos y eosinofilos de sangre periférica. El aumento de neutrofilos se produce apoximadamente a los 7 días de la infección, mientras que la eosinofilia aparece al 15 día de la primoinfección y alrededor del tercer día tras la reinfección. Trabajos anteriores, realizados por nosotros, mostraron que la expulsión del parásito, dado que el ratón no es un hospedador óptimo, se produce desde el día 7 al 14 en la primoinfección, mientras que cuando los animales son reinfectados dicha expulsión se produce entre el día 0 al 9 de la reinfección. Estos resultados, obtenidos en sangre periférica, son un indicador de la importancia de los neutrófilos y eosinófilos en la reacción inmunológica frente a los parásitos intestinales, pudiendo ser considerados como un criterio de la reacción antiparasitaria, aunque la auténtica reacción necesaria para la expulsión se produce en la "lámina propia" intestinal de la region del yeyuno, hábitat de *Hymenolepis diminuta*, lugar donde se encuentran los eosinófilos recubiertos de IgE e IgA específicas para el parásito.

Palabras clave: *Hymenolepis diminuta*, leucocitos, sangre.

Introduction

After infection of mice with *H. diminuta* an eosinophil response was demonstrated in the lamina propria of the intestine. Time of response was correlated with the rejection periods of the parasite (Van Der worst *et al.*[14]). The numbers of IgE bearing eosinophils after primary infection and the numbers of IgA bearing cells after reinfection were also correlated with the rejections periods. The highest peaks in the numbers of IgE and IgA bearing eosinophils were measured in the jejunum (Van Der worst *et al.*[13]) which is the habitat of the parasite (Turton[12]). The presence of IgE (Khalife *et al.*[6]) and of IgA

(Capron *et al.*[3]) on the eosinophils is an indication that these cells were activated by the infection.

Although, eosinophil responses have been demonstrated in most parasitic infections, no report has ever been made of an increased eosinophil response in peripheral blood as the result of an infection with the intestinal parasite *H. diminuta*.

In order to obtain this lacking information, the influence of a *H. diminuta* infection on the leukocytes in peripheral blood was evaluated.

Material and methods

Fifteen 6-week-old female Swiss albino mice were divided into 3 equal groups: a control, a primary infected group and a reinfected group. On day 0, the mice of the infected groups were orally infected with 10 cysticercoids by stomach tube. The animals of the reinfected group had already been infecte a first time on day -14.

Blood samples were collected without anticoagulant on days 3, 7, 11, 15 and 22 by orbita-punction. After collection the blood was used immediately to make smears. The leukocytes in the smears were stained after fixation (15 min with methanol) using the Giemsa-May Grunwald method: incubate for 3 min in May-Grunwald (Merck) and for 1 min in May-Grunwald (1/1 water, v/v), wash in water, incubate for 20 min in Giemsa (Merck, 1/25 water, v/v), wash in water, let differentiate for 10 min in water, dry at room temperature (20 min). Alfter this staining 200 leukocytes were differentiated in each smear, randomly.

All statistical analyses were performed on the arcsin transformed values. The homogeneity of the variances of the results was checked using BARTLETT's test. After annalysis of the variances, the significance of the differences was evaluated with STUDENT's t-test. The points of significance (P<0.05) are marked on the figures.

Results

The concentration of lymphocytes in blood (Fig. 1) of control animals was constant between 80 and 85% during the experimental period. After primary as well as after reinfection the concentration of lymphocytes was generally lower than in blood of control animals. However, the concentration was only significantly lower than the control values on day 3 after reinfection.

The concentration of neutrophils (Fig. 2) was constant (10-14%) in the blood of control animals. Three days after primary infection, the concentration was significantly lower than in control and reinfected animals. However, 7 days after primary infection the concentration was already significantly higher (19%) han in the control animals (12%). The concentration of the neutrophils after reinfection was similar to the concentration in control animals.

The concentration of monocytes (Fig. 3) in blood of control and infected animals varied between 3 and 5%. Significantly more monocytes than in the control animals were counted on day 11 after primary infection. On day 15 after reinfection the concentration of these cells was significantly higher than in control and primary infected animals.

The concentration of eosinophils (Fig. 4) in the blood of control animals did not increase above 2% in the course of the experiment. Fifteen days after infection significantly more eosinophils were present in the blood of primary infected mice than in the blood of control and reinfected animals. In reinfected animals the concentration was significantly higher than the concentration in the control and primary infected mice on day 3.

The concetration of basophils never exceeded 1 cell per 200 leukocytes in control animals and infection had no effect on the number of these cells. Statistical analyses on these results were not performed because in most of the samples, no basophils were found, among the 200 leukocytes differentiated.
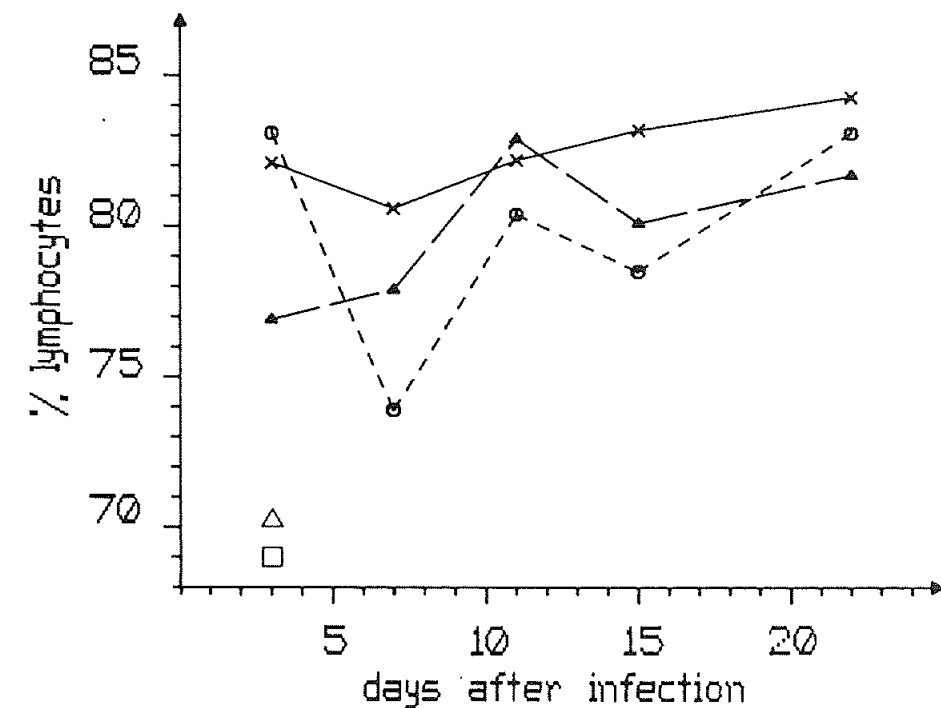

Fig. 1. The percentage of lymphocytes in peripheral blood of control (———), primary infected (- - - - -) and reinfected mice (– – – – –), different days after infection with *H. diminuta*. Significant differences (P < 0.05) between controls and infected mice (O), controls and reinfected mice (△) and between infected and reinfected mice (□) are indicated.
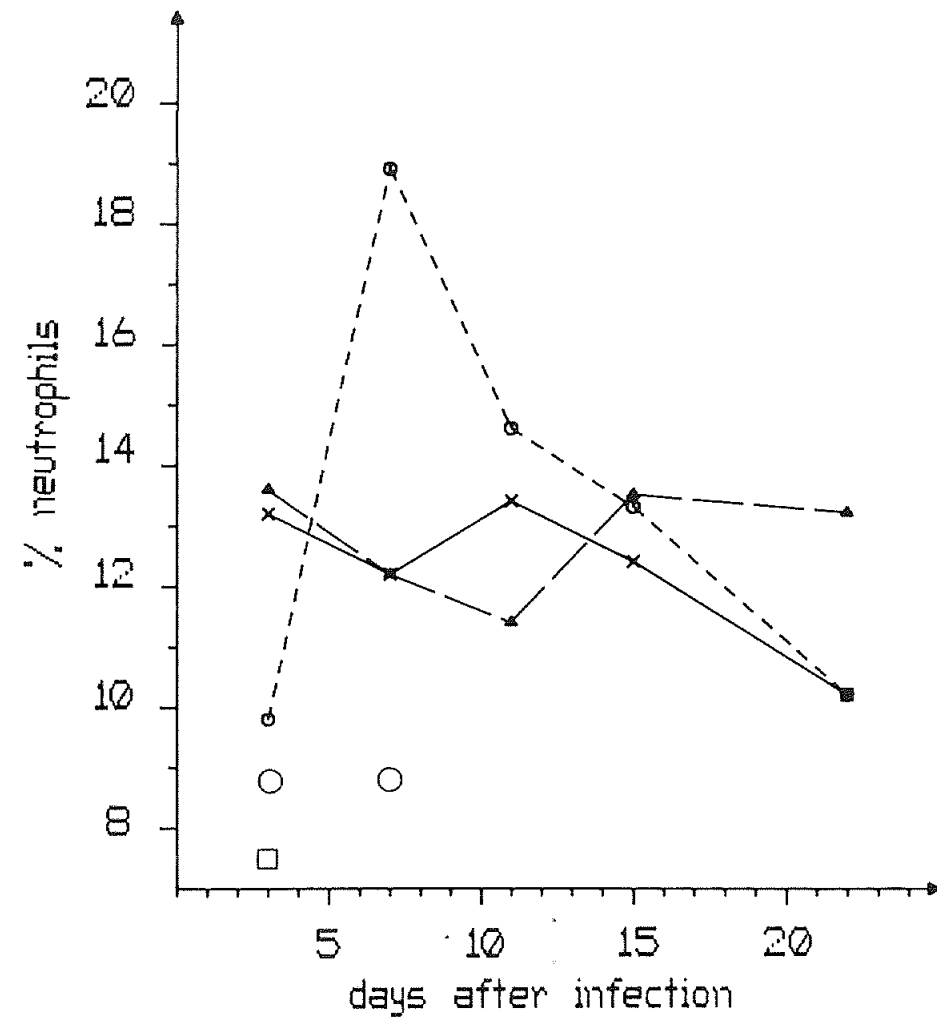
Fig. 2. The percentage of neutrophils in peripheral blood of control (————), primary infected (- - - - -) and reinfected mice (– – – – –), different days after infection with *H. diminuta*. Significant differences (P < 0.05) between controls and infected mice (O), controls and reinfected mice (△) and between infected and reinfected mice (□) are indicated.
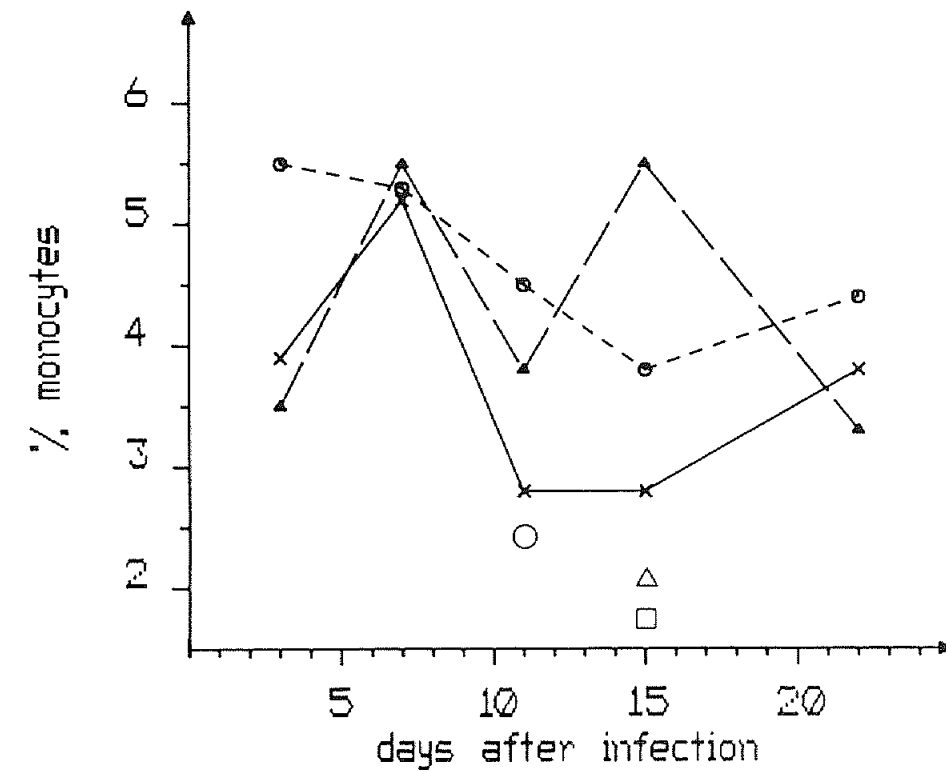

Fig. 3. The percentage of monocytes in peripheral blood of control (————), primary infected (- - - -) and reinfected mice (– – – – –), different days after infection with *H. diminuta*. Significant differences (P < 0.05) between controls and infected mice (O), controls and reinfected mice (△) and between infected and reinfected mice (□) are indicated.
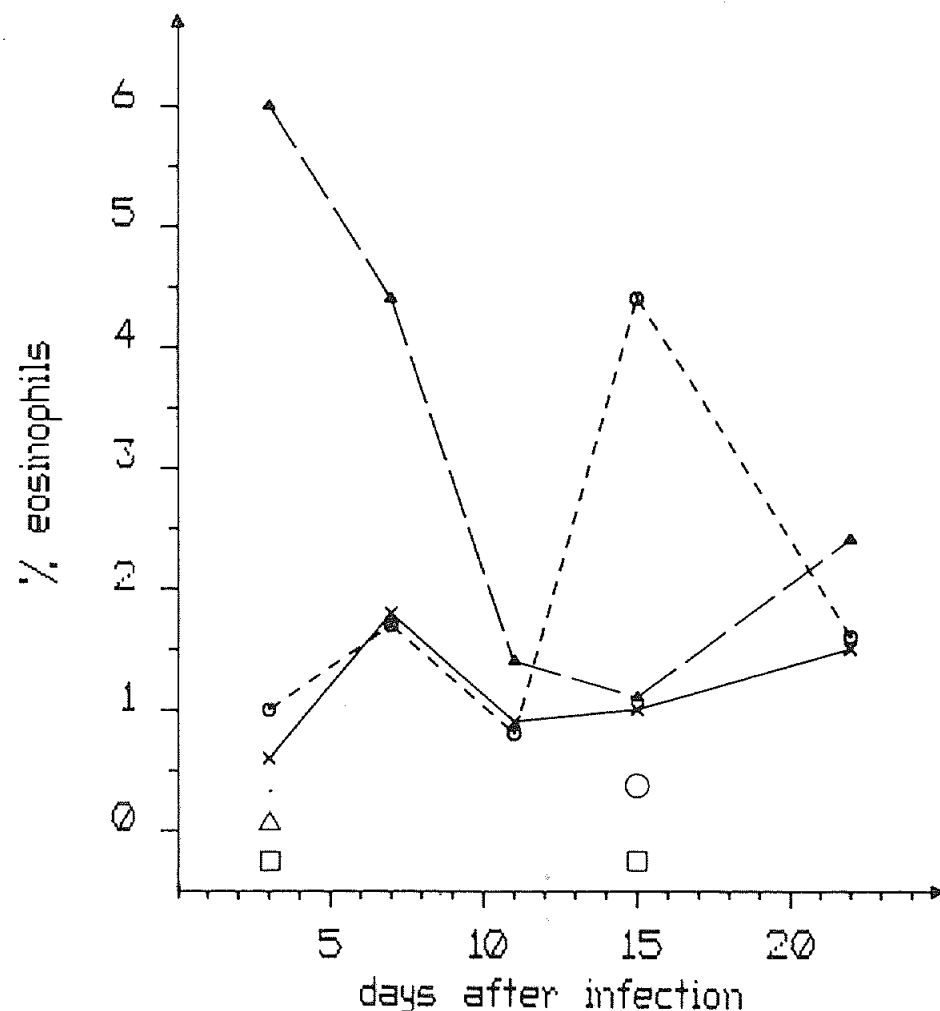
Fig. 4. The percentage of eosinophils in peripheral blood of control (————), primary infected (- - - -) and reinfected mice (– – – –), different days after infection with *H. diminuta*. Significant differences (P < 0.05) between controls and infected mice (○), controls and reinfected mice (△) and between infected and reinfected mice (□) are indicated.

Discussion

Except for the basophils, blood leukocytes show significant variations in answer to an infection with *Hymenolepis diminuta*.

The percentage of monocytes shows some significant differences in the course of infection. However the differences are small and can not be correlated with the rejection periods of the parasites.

The percentage of lymphocytes shows a significant decrease on day 3 after reinfection. This difference, together with the non-significant decreases after primary infection, are probably due to the fact that the data are represented as percentages. As a result the differences should rather be ascribed to increases of other leukocytes than to the decrease of lymphocytes.

One of the increases, which may be responsible for the decrease of lymphocytes on day 7 after primary infection, is the significant increase of neutrophils measured on day 7 after primary infection. This neutrophil increase can be explained considering the results reported by Larsh and Race[7], describing a major role for the neutrophils in the inflammatory reaction to *Trichinella spiralis* infections in mice. Moreover Bellamy and Nielsen[2] described that, as a result of antigen stimulation, neutrophils can migrate into the lumen of the small intestine. In the lumen they can disintegrate and liberate cytotoxic products. Mc Laren *et al.*[8] described the existence of such a "neutrophil cationic protein" which is cytotoxic to young schistosomula of *Schistosoma mansoni*.

The significant increases in the eosinophil concentrations on day 15 after primary infection and on day 3 after reinfection may be held responsible for the decrease of lymphocytes on the same days. These peaks in the eosinophil response are correlated with the rejection periods of *H. diminuta*. Although significant, the response is moderate as compared to other anti-parasitic responses, e. g. *Nippostrongylus brasiliensis* in rats

(10-16%; Ogilvie *et al.*[9]), and to *Ascaris suum* in mice (17%; Prokopic *et al.*[10]). The moderate eosinophilic response is characteristic for the immunological reactions against helminths in the intestine as stated by Atias[1]. Comparing the eosinophil concentration in the peripheral blood with the concentration in the intestinal wall (Van der Vorst *et al.*[14]), it is striking that the eosinophil concentration in blood reaches a peak on day 15 after primary infection, while in the intestinal wall the peak concentration is reached already on day 3. A similar reaction was demonstrated after a *Trichinella spiralis* infection in the rat (Ismail and Tanner[5]). These results are also in agreement with the general statement of Strath *et al.*[11], pointing out that the eosinophil concentration in the peripheral blood gives a poor indication of the eosinophil concentration in the tissues. After secondary infection the eosinophil response in blood reaches a peak on day 3.

It may concluded that neutrophils and eosinophils are involved in the immunological reaction to *H. diminuta*. However, the moderate eosinophil response and the absence of success to demonstrate IgE and IgA on the eosinophils in the blood in contrast to the eosinophil response in the intestine (Van Der Vorst *et al.*[13]), indicate that specific intestinal parasites evoke a local immunological reaction. The scope of this reaction in the intestine can be estimated by measuring the response in the blood, but one should be aware of the fact that this response is different and not always an equivalent picture of the local reaction.

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