

CYTOKINE RESPONSE IN THE INTESTINAL LAMINA PROPRIA OF MICE AFTER INFECTION WITH *FASCIOLA HEPATICA*

Philip J. van der Heijden,¹ Jan B.W.J. Cornelissen,¹ Elvera G. Breedland,² Huub F.J. Savelkoul,² and Andre T.J. Bianchi¹

¹Department of Immunology Central Veterinary Institute, Lelystad, and ²Department of Immunology Erasmus University, Rotterdam, The Netherlands

INTRODUCTION

It has become evident, that the isotype distribution of the immune response, is regulated by the cytokine profile in the microenvironment where the immune reaction is induced.¹ For example, IgG2a responses are induced predominantly at higher levels of IFN-gamma and IL2, cytokines secreted by Th1-type cells. In contrast, IgG1, IgA and IgE responses are induced predominantly at higher concentrations of IL-4 and IL-5, cytokines secreted by Th2-type cells. Therefore, to study the regulation of the immune response and to be able to manipulate these responses, quantitation of locally secreted Th1- or Th2-type cytokines is relevant. Our research is focused on the regulation of immune responses against infectious agents, especially at the mucosal level. In this paper, we used as a model mice infected with the parasite *Fasciola hepatica*. Parasitic infections are often accompanied by high IgE responses and are therefore thought to be correlated with an increased level of IL-4 and IL-5 secretion. We analyzed mRNA isolated from intestinal lymphocytes for IFN-gamma, IL-4 and IL-5 message after primary and secondary infection with *Fasciola hepatica*. The results were compared with total IgE levels found in serum after infection.

MATERIALS AND METHODS

Mice

Female C3H/HeN mice were purchased from Charles River (Germany) and housed under conventional conditions. Mice were used at the age of 12-20 weeks.

Infection

One group of mice (group 1, n=10) was infected orally (day 0) with 2 metacercariae and challenged orally (day 34) with 50 metacercariae. A second group (group 2, n= 10) was primed orally with 50 metacercariae (day 34). A third control group (group 3, n= 10) was left untreated.

Detection of Cytokine Message in Lamina Propria Lymphocytes

Five mice per group were killed with carbon dioxide 24 h after the last infection (day 35). Lamina propria lymphocytes (LPL) were prepared as described.² RNA was extracted from LPL using guanidinium isothiocyanate in combination with cesium chloride density gradient centrifugation according to the method of Chirgwin *et al.*³ The isolated RNA was

reverse transcribed into cDNA by using reverse transcriptase.⁴ Subsequently, 0.1 µg of cDNA was amplified by 35 cycles in PCR using Taq polymerase and primers specific for IL-4, IL-5 and IFN- γ combined with primers for a house-keeping gene (HPRT). The PCR products were visualized by running agarose gel electrophoresis and ethydiull bromide staining. Gels were photographed and the intensities of the bands were quantified by densitometrical analysis.

Quantitative Analysis of Serum IgE Levels

Blood samples from five mice per group were collected on days 0, 7, 10, 14, 35, 42, 49. The concentration of total IgE in serum was quantified by an isotype-specific ELISA, as described.⁵

RESULTS

Detection of Cytokine Message in Lamina Propria Lymphocytes

Lamina propria lymphocytes were isolated from all mice 24 h after the last infection (day 35). Cytoplasmic RNA was extracted from 10^7 lamina propria lymphocytes. Aliquots of mRNA were then reverse transcribed and the resulting cDNA subjected to 35 cycles of PCR using pairs of oligonucleotide primers specific for one type of mRNA combined with primers for the house-keeping gene. The cDNA-PCR amplified products were subjected to agarose gel electrophoresis. Fig. 1 shows a typical result after electrophoresis of the amplified HPRT and IL-5 products. In lane 1 and 2, specific bands for the amplified cDNA of IL-5 are visible, whereas in lane 3 a negative result is shown. In lanes 1, 2 and 3, the HPRT product is visible. In lane 4, the apparent decrease in the density of the IL5 band is an artefact because the density of the HPRT band, the internal control probe, was also decreased. Table 1 shows the relative densities of the amplified cDNA of IL-4, IL-5 and IFN-gamma of all three groups. The results suggest that after a primary infection the message for IL-4 and IL-5 increases, whereas the message for IFN-gamma does not change. After a secondary infection the message for IFN-gamma decreases, whereas the message for IL-4 and IL-5 equalled those of the control group.

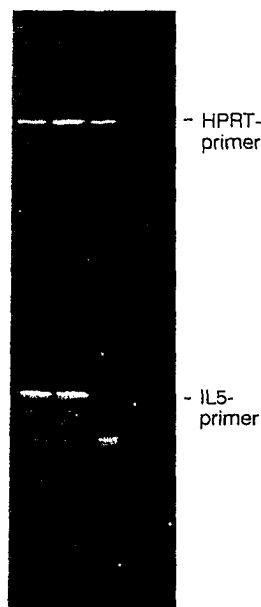


Figure 1. PCR-amplified cDNA fragments, generated using oligonucleotides specific for IL-5, separated by agarose gel electrophoresis. HPRT-specific oligonucleotides were used to determine the assay validity.

Table 1. Analysis of message for IFN-gamma, IL-4 and IL-5 by reversed PCR in isolated intestinal lamina propria cells after primary (group II) or secondary (group I) infection with *Fasciola hepatica*. Non-infected mice were used as a control (group III).

	Group I	Group II	Group III
IL-4	+/-	+	+/-
IL-5	+/-	+	+/-
IFN-gamma	+/-	++	++

+/- bands hardly or not visible

+ bands visible

++ clear bands visible

Quantitative Analysis of Total IgE in Serum

IgE concentrations were determined in the sera of group I,II and III. The results demonstrated that serum IgE levels in *Fasciola hepatica* infected mice increased significantly (Fig. 2). The total IgE levels after primary infection (Group I, day 35) exceeds those after secondary infection (Group I, day 49). A plateau level was reached at 67 ,ug/ml 35 days after primary infection with 2 metescaraine.

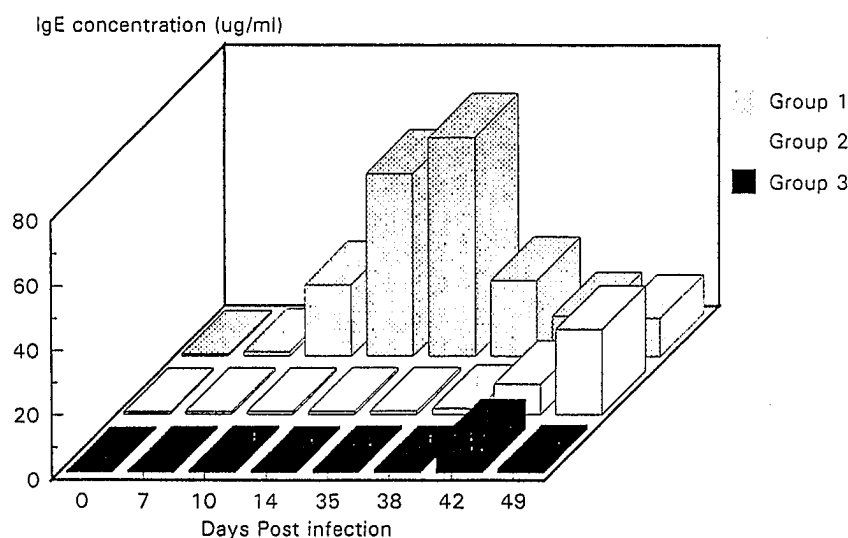


Figure 2. IgE levels in C3H/He mice infected with *Fasciola hepatica*. Group 1: hltected twice; Group 2: Infected once; Group 3: Control Group.

DISCUSSION

In this study, we demonstrated that it is possible to analyze mRNA in isolated lamina propria lymphocyte suspensions for message for a specific cytokine by reversed PCR. Therefore, this approach can be used to study the mechanisms regulating immune response at the mucosal level. As the yield of isolated mRNA can vary, we used an internal control probe, HPRT, to be able to discriminate artifacts in the performance of the assay. The results on the effect of infection with *Fasciola hepatica* on cytokine profiles in the intestinal mucosa described in this paper are based on very limited studies. First, we only measured mRNA message 24 hours post infection and did not yet study the kinetics of these responses. Moreover, the technique used does not allow quantitative measurements of actually secreted cytokines. Therefore, all conclusions based on these results 011 probable

mechanisms of immune regulation must be considered very carefully. However, even with these limited results, the total IgE-levels could be correlated with the PCR results. Enhanced message for IL-4 and IL-5 after primary infection coincided with an increase in IgE levels. After secondary infection, the increase in IgE was much lower and correlated with a normal level of message for IL-4 and IL-5. However, the balance between Th1 and Th2 cytokines changed in favor of Th2, compared to the control group, as the message for IFN-gamma was decreased.

As stated above, the PCR does not give quantitative results on actually secreted cytokines. Therefore, we plan to enumerate the cytokine secreting cells in isolated lamina propria lymphocytes by cytokine specific spotassays. These results, together with the results of the techniques used in this study, will contribute greatly to our understanding of the regulation of local immune responses.

REFERENCES

1. T. R. Mosmann and R. L. Coffman, *Immunol. Today* 8:223 (1987).
2. P. J. van der Heijden and W. Stok, *J. Immunol. Methods* 103: 161 (1987).
3. J. J. Chrigwin, A. E. Przbyla, R. J. MacDonald, and W. J. Rutter, *Biochemistry*, 18:5294 (1979).
4. L. J. Murray, R. Lee, and C. Martens, *Eur. J. Immunol.* 20:163 (1990).
5. H. F. J. Savelkoul, P. W. C. Soeting, J. E. de Josselin-de Jong, and S. S. Pathak, *J. Immunol. Methods* 116:277 (1989).