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VOOR PARASITOLOGIE

NETHERLANDS SOCIETY  
FOR PARASITOLOGY

G837/88

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Geachte Heer de Geus,

Hierbij heb ik het genoegen U de abstracts van onze  
voorjaarsvergadering toe te sturen voor plaatsing in het T.G.M.

Zoudt U ze willen plaatsen onder de kop:

NETHERLANDS SOCIETY FOR PARASITOLOGY

Spring meeting, including a symposium titled:  
Eosinophils and Immunoglobulin E: Immunobiology and  
function in parasitic and allergic respiratory disease.  
Held on April 7, 1988, Utrecht, The Netherlands

De volgorde van de abstracts is met potlood rechtsboven  
aangegeven.

Met hartelijke dank en vriendelijke groet,



Dr.F.H.M.Borgsteede, secretaris NVP

Onder auspiciën van de "Nederlandse Vereniging voor Parasitologie"

Titel:

EOSINOPHILS AND IMMUNOGLOBULINE E: IMMUNOBIOLOGY AND FUNCTION IN PARASITIC AND ALLERGIC RESPIRATORY DISEASES

Coördinator: J. Buijs

Voorzitters: A.M. Deelder

(1e gedeelte Deelder voorzitter, Ruitenbergh co-voorzitter)

(14.30 - 15.50)

E.J. Ruitenbergh

(2e gedeelte Ruitenbergh voorzitter, Deelder co- voorzitter)

(15.50 - 17.00)

14.30 - 14.50 Aalberse, R.C.

IgE and interleukin-4 in allergic and parasitic diseases

14.50 - 15.30 McLaren, D.J.

The role of effector cells in immunity to trematode infections

15.30 - 15.50 Roos, D., M.Yazdanbakhsh, C.M.Eckmann

Schistocidal action of eosinophils

15.50 - 16.10 Monchy, J.G.R. de

Eosinophils and IgE in allergic respiratory disease

16.10 - 16.30 Parmentier, H.K., H.van Loveren, E.J.Ruitenbergh

Interference with intestinal inflammation during a *Trichinella spiralis* infection in mice does not affect worm expulsion

16.30 - 16.50 Buijs, J.

Eosinophils in nematode infections

16.50 - 17.00 Concluding remarks

SCHOONE, G.J., G.S. LIGTHART, C.C.M. KROON, W.J. TERPSTRA, G.J.J.M. VAN EYS  
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Identification of Leptospira Strains by In Situ Hybridization with non-radioactive recombinant DNA probes.

Leptospira can be classified to serovar level by antisera or monoclonal antibodies. For routine purposes this level is sufficient. However in a number of cases immunological techniques do not differentiate between pathologically different strains. Therefore, we investigated the potential of DNA techniques to solve this problem. Restriction enzyme analysis shows considerable differences between leptospiral strains. Unfortunately, this method is time consuming (culturing the leptospira) and relatively expensive. In situ hybridization with total DNA probes showed extensive cross hybridization. Therefore, genomic libraries were constructed and strain specific clones were picked after differential hybridization. These clones were further characterized by dot blot and Southern blotting. Finally the clones were applied as biotin labelled probes in an in situ hybridization assay. Recombinant probes give a good signal although not as strong as total DNA probes. However, recombinant probes showed high specificity. A specific hybridization in combination with recognizable morphology offers the possibility of simultaneous detection and characterization of the leptospira.

We, therefore, conclude that carefully selected recombinant probes might provide tools for routine diagnosis of infecting organisms by in situ hybridization.

KROON, C.C.M., G.S. LIGTHART, G.J. SCHOONE, W.J. TERPSTRA and G.J.J.M. VAN EYS  
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Characterization of Leishmania Parasites by DNA Hybridization.

Leishmania parasites can not be characterized on the basis of microscopical observation or culture requirements. Despite the development of monoclonal antibodies immunological characterization has proven to be unreliable. So, the iso-enzyme test has been the method of choice for classification of Leishmania parasites. The disadvantages of this method are the necessity to culture the parasite and the increasing complexity of the classification with the addition of new iso-enzymes. Recently, DNA technology has provided new opportunities to tackle the problem of Leishmania characterization. Investigators have focused on kinetoplast DNA since this would provide more than 1000 copies/organism of identical minicircles. Unfortunately, variations of the minicircle DNA occur within species. This raises questions about the applicability of this type of DNA for characterization of Leishmania parasites. Therefore we have chosen to develop DNA probes selected from genomic and cDNA libraries. Our results so far show that these probes are capable to differentiate between complexes and strains of Leishmania parasites. Most probes cross react with DNA of several Leishmania strains but will show different patterns on Southern blots, whereas some probes seem to be strain specific in different hybridization assays. Southern blots are, at this moment, the method of choice but in the future they might be replaced by dot blot or in situ hybridization assays with truly strain specific probes.



RC Aalberse  
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IgE and Interleukin-4 in allergic and parasitic diseases.

From the allergic point of view parasitic disease is a most interesting "experiment of nature": it may help to elucidate the cause of the abnormal regulation of the humoral response in atopy. The most striking aspect of the IgE system is perhaps not the relatively increased levels in atopy and parasitosis, but the very low levels under normal conditions. It is at the moment unclear why this particular immunoglobulin isotype is so rarely expressed. What makes production of IgE different from production of other isotypes? One attractive hypothesis is that this phenomenon is related to the almost ubiquitous presence on lymphocytes of an IgE-binding protein CD23 (a low-affinity IgE-receptor distinct from the high affinity IgE receptor on mast cells and basophilic leukocytes), which seem to be involved in B cell growth/differentiation. The expression of this receptor is markedly enhanced by the interleukin IL-4, which is also a potent inducer of IgE synthesis. A possibility would then be that lymphocytes with a high percentage of their IgE receptors blocked by IgE would be halted in their differentiation (autocrine inhibition). Cells that do not synthesise IgE would normally be hardly affected. Under certain conditions the production of IL-4 is increased (parasitosis, atopy), which results in an increased expression of IgE-receptors, overcoming the autocrine inhibition of IgE-producing cells.

R.C. Aalberse

R.C. Aalberse

IgE and Interleukin-4 in allergic and parasitic diseases.  
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X

Eosinophils and Immunoglobulin E: Immunobiology  
and function in parasitic and allergic respiratory  
disease

IgE

IL-4

ε receptor

MCLAREN, D.J.

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The Role of Effector Cells in Immunity to Trematode Infections.

Considerable evidence has now accumulated to indicate that effector cells play a crucial role in protective immunity against trematode parasites. Moreover, contrary to data obtained from in vitro studies, which suggest that invading skin stage larvae are primary targets of such mechanisms, there is now good reason to believe that cellular cytotoxicity in vivo, may operate in a variety of other organs against juvenile, or even older worms. In many cases the skin is indeed the first physical and immunological barrier to be encountered by the parasite. Here, eosinophils, neutrophils, mast cells, basophils and macrophages have all been shown to participate in primary and/or secondary responses. Evidence implicating each cell type (accrued from histology, leucocyte ablation and passive transfer studies, or the use of selective immunomodulatory agents etc.) will be presented, together with observations on the interactive or regulatory role of humoral factors, or other components of the immune system. Possible mechanisms of cell-mediated parasite damage and killing will be proposed. Data showing that the same or different responses may participate in immune attrition at the level of the lungs and/or liver will also be discussed, as will the complication imposed by parasite evasive strategies. Mention will also be made of the interactive role of the immune response in promoting effective chemoprophylaxis. The present paradox between data obtained from in vitro and in vivo models of protective immunity will be highlighted.

DIANE J. MCLAREN

Dr Diane J. McLaren

The Role of Effector Cells in Immunity to Trematode Infections.  
Medical Research Council of Great Britain

X

Eosinophils and Immunoglobulin E: Immunobiology  
and function in parasitic and allergic respiratory  
disease

EFFECTOR CELLS

TREMATODES

IMMUNITY

ROOS, D., M. YAZDANBAKHSH and C.M. ECKMANN

Central Lab. Neth. Red Cross Blood Transf. Service and Lab. Exp. and Clin.

Immunology of the University of Amsterdam

### Schistocidal action of eosinophils

Eosinophilic granulocytes contain at least two different mechanisms of parasite killing: toxic proteins stored in granules (MBP, ECP, EDN) and toxic oxygen metabolites produced by an  $\text{NADPH}:\text{O}_2$  oxidoreductase and subsequent reactions. Reports about the importance of each of these systems are contradictory. We have evaluated the toxicity of purified major basic protein (MBP) and eosinophil cationic protein (ECP), as well as superoxide ( $\text{O}_2^-$ )/hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), towards schistosomes of *S.mansoni*. Moreover, we have also studied the effect of combinations of these agents. We found MBP and ECP to be toxic at concentrations of  $6 \times 10^{-5}$  M and  $4 \times 10^{-6}$  M, respectively. Cytoplasts made from eosinophils (without nucleus and granules) produced  $\text{O}_2^-$  and  $\text{H}_2\text{O}_2$  upon contact with opsonized schistosomes, but did not kill them. A clear synergy in the toxicity was observed when ECP ( $10^{-6}$  M) was combined with eosinoplasts, but not when MBP ( $1-20 \times 10^{-6}$ ) was combined with these cytoplasts. To study the role of stable oxygen metabolites, we added taurine-chloramine (T-Cl) to schistosomes. Because we found a strong preference of eosinophil peroxidase for bromide over chloride, we also tested taurine-bromamine (T-Br). Both taurine derivatives were found to be highly toxic for schistosomes. These results indicate that both oxygen-dependent and -independent mechanisms can be used by eosinophils to kill schistosomes. Together, these mechanisms can produce synergistic effects for highly efficient killing.

D. Roos

M. Yazdanbakhsh

C.M. Eckmann

D. Roos, M. Yazdanbakhsh, C.M. Eckmann

Schistocidal action of eosinophils

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Eosinophils and Immunoglobulin E: Immunobiology and function in parasitic and allergic respiratory disease

Eosinophils

Peroxidase

Schistosomes



MONCHY, J.G.R. DE

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Eosinophils and IgE in allergic respiratory disease.

Until recently, allergic bronchial asthma was considered as a problem of muscular spasm in response to mediators derived from IgE-mediated mast cell degranulation. Microscopic studies of the bronchial mucosa in asthmatic patients as well as a number of experimental studies following allergen challenge, however, suggest that an important local inflammatory component is also present in these patients. Bronchial alveolar lavage (BAL) of patients 6-7 hours after allergen (house dust mite) challenge showed an increase in eosinophils in those patients with combined early and late (LAR) reactions. No significant increases in other cell types were found in the BAL of these patients.

In skin test studies most authors found mixed cellular infiltrates with a certain preponderance of eosinophils occurring prior to the development of late cutaneous reactions (LCR). The LCR could be transferred from one subject to another by IgE-containing serum. When the serum was heat-inactivated, not only the transmission of the early reaction (ECR), but also of the LCR was inhibited. Dolovich showed that the LCR could be induced by injection of anti-IgE antibodies. It is tempting to speculate that the eosinophil infiltration occurring during the late allergic reaction is mediated by IgE-dependent mast cell degranulation since mast cells can produce chemoattractants for eosinophils.

J.G.R. de Monchy

J.G.R. de Monchy

Eosinophils and IgE in allergic respiratory disease

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Eosinophils and Immunoglobulin E: Immunobiology  
and function in parasitic and allergic respiratory  
disease

Eosinophils

IgE

Allergy

PARMENTIER, H.K.<sup>1</sup>, H. VAN LOVEREN<sup>2</sup> and E.J.RUITENBERG<sup>1 2</sup>.

<sup>1</sup> Department of Immunology, Veterinary Faculty, University of Utrecht,

<sup>2</sup> Laboratory for Pathology, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.

Interference with intestinal inflammation during a *Trichinella spiralis* infection in mice does not affect worm expulsion.

Intestinal nematode infections (e.g. *Trichinella spiralis*) in the rodent gut are characterized by a T-cell dependent cellular infiltrate comprising (mucosal) mast cells, eosinophilic granulocytes, IgA secreting plasma cells and increasing activity of mucus secreting cells. Since intestinal inflammation is most conspicuous at the time when the adult worms are expelled from the gut, it was speculated that non specific inflammatory cells (releasing toxic products, perhaps while adhering to the parasite) are responsible for worm expulsion, either by killing or damaging adult worms, or inducing a unfavorable environment in the gut forcing the worms to leave the jejunal epithelium. Though in vitro non-antigen specific inflammatory cells e.g. mast cells and eosinophils can be armed with immunoglobulins or T-cell factors to become cytotoxic (eosinophils) or release anaphylactic modulators (mast cells), their in vivo role in primary worm expulsion remains to be established.

Recently, it was found that the mast cell- and eosinophil rich inflammation in the gut of *T.spiralis* infected mice is regulated by similar mechanisms as operating in delayed-type hypersensitivity in the skin of mice due to contact sensitizers. Antigen specific T-cell factors are thought both to initiate and to suppress the intestinal cellular infiltrate. Interference with the activity of the *T.spiralis* specific T-cell factors both immunologically, and by treatment of animals with drugs, resulted in a suppression of intestinal inflammation, exemplified by reduced numbers of mast cells and eosinophils. In contrast to this, the rate of adult worm expulsion was not affected.

Since worm expulsion could occur with very low numbers of inflammatory cells in the gut, we speculate that immune effector mechanisms responsible for primary worm expulsion are independent of local hypersensitivity responses.

H.K.Parmentier

H. van Loveren

E.J.Ruitenbergr

Parmentier, H.K., H. van Loveren and E.J.Ruitenbergr

Interference with intestinal inflammation during a *Trichinella spiralis* infection in mice does not affect worm expulsion.

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Immunoglobulin E and eosinophils: its biological function in parasitic and allergic respiratory diseases.



J.Buijs and E.J.Ruitenber

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Eosinophils in nematode infections

Nematode infections in small laboratory rodents induce eosinophilia in blood and tissues. It was suggested that eosinophils possessed direct toxic properties towards the vulnerable (i.e. larval) stages of parasites. Eosinophils contain toxic proteins (e.g. major basic protein (= MBP) and lysosomal peroxidase (EPO) which catalyzes the generation of the oxidant hypochlorite ( $OCl^-$ ) from chloride ions and hydrogen peroxide. The toxicity of eosinophils and some of its products were assayed using the newborn larvae of Trichinella spiralis and stage two larvae of Toxocara canis (TcL<sub>2</sub>) as targets. Trichinella spiralis causes a transient inflammation of the gut in which eosinophils are prominent. T.canis causes the larva migrans syndrome and induces eosinophilic granuloma's in lung and liver of mice for a prolonged period. Results were compared with the results obtained using polymorphonuclears (PMN's) and peritoneal macrophages as effector cells. Using NBL it was shown that 1) macrophages are better killer cells than eosinophils 2) that in the presence of myeloperoxidase (MPO) from PMN's NBL killing is more efficient than in the presence of EPO and 3) that MBP, apart from the cytotoxic properties, served as scavenger for  $OCl^-$ . None of the systems were effective when TcL<sub>2</sub> were used as targets.

J.Buijs

E.J.Ruitenber

J.Buijs

Eosinophils in nematode infections

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eosinophils

nematodes