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EOSINOPHILS AND MONONUCLEAR CELLS AS EFFECTOR CELLS IN A *TRICHINELLA SPIRALIS* INFECTION; CELL BIOLOGICAL AND BIOCHEMICAL ASPECTS AND THE USE OF BIOLOGICAL RESPONSE MODIFIERS

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Introduction

A host invaded by parasites is only partially successful in diverting the invasion. Although a number of the parasites never reach maturity, the remaining number is often large enough to confront the host with serious problems. Especially those parasites which pass part of their life cycle in tissues outside the gut, may cause serious damage to various organs. In several parasitic infections superinfections may occur, indicating that the host fails in building up a solid immunity against reinvasion. Examples are easily found in parasitic helminths as well as in parasitic protozoan infections. The latter phenomenon may be due to the fact that parasites, in their struggle for survival in ancient times, learned how to modulate the host's defence system to their own advantage.

This presentation discusses the host's response on a *Trichinella spiralis* infection. The experimental animals used are small laboratory rodents. This model is favoured as the *T. spiralis* passes its full lifecycle in one host, causes a.o. histopathological changes in the gut, inflammatory reactions of the parasitized tissues and immune suppression. The cells involved in the host's defence, their actions and possible effectors mechanisms will be dealt with. Finally a possible approach for the research in vaccine development in relation with parasitic diseases will be considered.

The parasite

Infection with the nematode *Trichinella spiralis* in man generally occurs by eating infected raw or undercooked pork or boar. The infected meat contains encysted muscle larvae (ML) which are enzymatically

freed from their cysts during passage through the stomach to the small intestine. In the experimental model the animals are orally infected with muscle larvae. In the gut the larvae mature to adult worms (AW). The females penetrate the gut wall without passing the basal membrane and produce after copulation, live larvae, i.e. the new born larvae (NBL). The NBL migrate via various ways to the striated muscles where they encyst. These ML thus stay alife for a very long time. In small laboratory rodents the intestinal phase lasts for 12-18 days after which the worms are expelled by host action. The migrating phase starts with the production of NBL (6 days p.i.) and lasts as long as adult females are present in the gut. The muscle phase starts with the arrival of the NBL in the striated muscles (from 7-8 days p.i.).

The host's response

A number of ingested larvae never reach maturity. This is probably caused by a combination of factors, as low viability of a number of the larvae and host action. The worms reaching maturity cause transient histopathological reactions of the intestine and regional lymphoid tissues. The villus/crypt ratio in the jejunum is reduced from the usual 4/1 to 1-2/1 (Ruitenberg et al., 1977); increase of the mitotic index of epithelial cells is observed; the numbers of inflammatory cells in the lamina propria are increased. In particular eosinophils (eo's) (Ruitenberg and Buys, 1980; Ruitenberg et al., 1977); intestinal mast cells (IMC) and its intraepithelial counterpart, the globule leucocyte (GL) show remarkable proliferation (Ruitenberg et al., 1979). This is accompanied by oedema of the lamina propria, the submucosa and the smooth muscle layers. In the mouse the increase in eosinophils is reflected in the peripheral circulation (Ruitenberg et al., 1977). Eosinophilia as high as 25-30% of the total leucocyte number may occur at 3 weeks p.i. In the rat the relative number of eo's in the circulation does not pass the 5-7%. However, in the bone marrow 25-30% of the total of nucleated cells (erythropoesis included) are made up of eo's at 10-15 days p.i. (unpublished results). Stage specific antibodies are produced, starting with anti-ML-antibodies (ab) at 6 days p.i. followed by anti-AW-ab at 8 days p.i. and anti-NBL-ab at 12 days p.i. Anti-ML-ab are being produced during a long period, whereas ab-against AW and NBL decline rather rapidly falling to very low titres at 40 days p.i. (Mackenzie et al., 1978). During encystation of the migrating larvae an inflammatory reaction at the sites of developing cysts is observed. Although the inflammatory cells mainly exist of mononuclear cells, eosinophils also are clearly present (Gustowska et al., 1980). The host response is largely dependent on the presence of T-cells

(Vos et al., 1983; Ruitenberg and Elgersma, 1976). Congenitally athymic small rodents (mice and rats) do not show any of the above mentioned histo-pathological changes with the exception of the reduction in villus/crypt ratio. This reduction however may be caused by mechanical damage to the epithelial lining during penetration of the females. This mechanically caused damage may stimulate natural repair by speeding up epithelial mitoses. There is also very moderate eosinophil response in the athymic animals upon a *T. spiralis* infection (Gustowska et al., 1980).

The operational cells of the host's immune system

The cell populations involved in the host's defence not only concern the T- and B-cell series but also the inflammatory cells as close operation exists between the two systems. A central position takes the macrophage (Mφ). The Mφ is generally considered as forming part of the inflammatory cell series. Mφ's become activated a.o. by the presence of foreign organisms which they either phagocytize or bind to the outer membrane (Buys et al., 1980; Ruitenberg et al., 1983). The interaction induces membrane perturbation including the activation of membrane-bound receptors and enzymes. This results in the intracellular production of a number of substances toxic for organisms present in phagolysosomes or, after exocytosis, for cell surface bound organisms.

However, by now, it is a well known fact that activated Mφ's, also release interleukine-1 (IL-1) a very potent activator of T-helper cells and of resting B-cells. Both cell populations respond on IL-1 by extensive proliferative activity (Palacios, 1982). The T-helper cells release during proliferation lymphokines as IL-2, IL-3 (Yung et al., 1981) and Interferon-γ (IF-γ) (Nathan et al., 1984). IL-2 activates the majority of the T-cell subpopulations (Palacios, 1982). IL-3 activates also particular T-cell subpopulations and induces formation and maturation of mast cells (Ihle et al., 1982). IF-γ induces on antigen presenting cells (APC) membrane expression of Ia antigens, molecules encoded for by Ir genes of the major histocompatibility complex (MHC) (Ezekowitz and Gordon, 1982). In order to become functional immune cells activation by the specific antigen is essential as only than the specific antigen receptors come to expression. Responsiveness of the participating cells depends on dual interaction i.e. the specific antigen receptors and the non-specific lymphokine receptors. B-cells which activation is also initiated by IL-1, mature into Ig secreting plasma cells in close cooperation with T-cells and APC's. Here too, activation is based on the dual recognition of antigen specific and antigen non-specific receptors (Staruch and Wood, 1984).

One of the enzymes activated during membrane perturbation is phospholipase-A2. Phospholipase-A2 catalyzes the hydrolysation of the bonds with which arachidonic acid is connected to certain membrane phospholipids. The arachidonic acid is liable to be processed by two different enzyme systems, i.e. the cyclooxygenase pathway with as final product prostaglandins or the lipoxygenase pathway in which different leukotrienes are the final products (König, 1982). Leukotriene B4 (LTB4) is known as a potent chemotactic factor of eo's and polymorphonuclear neutrophils (PMN's) which explains the influx of granulocytes and especially eosinophils into the gut. A possible succession of actions to attract eosinophils to the parasitized gut may be as follows: During the T-cell independent phase of eo response parasitic antigens are directly responsible for eo proliferation in the bone-marrow, in this case Mφ-derived LTB4 is responsible for chemotactic attraction. The late phase in eo response is supposed to be initiated by a T-cell factor. Chemoattraction is cared for by various factors among which C_5-b and chemoattractants derived from mucosal mast cells.

Actions of the different operational cells taking part in the host's defence system

Once the operational cells are fully matured effector mechanisms have developed which upon contact with parasite antigens become functional. The different types of effector cells react in a fashion characteristic of their kind. Activation of the participating cell populations often depends on the presence of one another or on one another's products. Activated B-cells become Ig secreting plasmacells after antigen presentation by APC's in the presence of T-helper cells (Julius, 1982). The secreted Ig's mediate adherence of inflammatory cells to the parasite surface (Buys et al., 1980; Ruitenberg et al., 1983). IMC proliferation depends on IL-3 produced by T-helper cells (Ihle et al., 1982). IMC activation occurs after interaction of the FcΣ receptors with IgE and the specific antigen (Ishizaka et al., 1970) or possibly with a T-cell derived mast cell specific factor (Van Loveren et al., 1983). IMC release a.o. histamine and a number of enzymes and produce prostaglandin and leukotrienes (LT's Kennerly et al., 1979). Histamine activates T-suppressor cells (T's) by interacting with the T's H_2 receptors and induces vasodilatation by interacting with the H_1 receptors on endothelial cells of the capillaries (Rocklin et al., 1979). IMC release also an eosinophil chemotactic factor responsible for the eosinophil influx (König, 1982). Eosinophils release histaminase which inactivates histamine and arylsulfatase-B which inactivates the slow reacting substance of anaphylaxis, both are IMC mediators. In "in vitro" assays it was demonstrated that

eosinophils during adherence to parasite surfaces release major basic protein (MBP), the major protein of the granule's core (Gleich et al., 1973). Together with the MBP peroxidase positive material is released and both are layered over the parasite's surface (McLaren et al., 1977). The MBP possess toxic properties towards the NBL as are the reacting oxygen metabolites produced by peroxidase directed reactions (Buys et al., 1984).

A way by which T-suppressor cells exert immune suppressive activity is by absorbing IL-2 thus inhibiting the activation of the majority of T-cell subpopulations (Palacios, 1982). It thus appears that under normal conditions the inflammatory and immune reactions in the gut following a *T. spiralis* infection are probably a self-limiting process. Despite this very intricate network of reactions the host's defence is unable to prevent the migration of NBL to the striated muscles. However, the defence is capable to limit the presence of AW in the gut to 18 days or less.

Effector mechanisms of inflammatory cells

The inflammatory cells possess Fc receptors for various Ig's i.e. Mφ's Fcγ and FcΣ; eo's Fcγ and FcΣ; PMN's Fcγ; all three have receptors also for complement (C'). Specific anti-*T. spiralis* ab or C' form a ligaud between cell and parasite surfaces. From an extensive "in vitro" study in which the effect of adherence of the various inflammatory cells to the different parasite stages was investigated it became evident that although adherence occurred to all three stages only the NBL were liable to get killed. Ab mediated the adherence of the various cells to all parasite stages whereas C' mediated adherence to mainly the muscle larvae did not induce any damage (Ruitenberg et al., 1983). With regard to the NBL the presence of immune serum proved to be essential for the induction of the larvicidal effect. A surprising observation was that in the *T. spiralis* model the eosinophils killed the NBL less effective than PMN's and Mφ's. Activated Mφ's and bone marrow PMN's killed about twice as many NBL as activated eosinophils (Ruitenberg et al., 1983). This observation differed from the results obtained with the *Schistosoma mansoni* model, in which the eo's were the main killer cells of the schistosomula (Butterworth et al., 1975).

In order to study the mechanism by which inflammatory cells kill their target the metabolic status of the cells was investigated. In the lumanol amplified chemiluminescence method the production of reactive oxygen metabolites was studied. Cells were stimulated with phorbol myristate acetate (PMA) in vitro after which the release of photons was counted with a scintillation counter. It was found that when cells were ma-

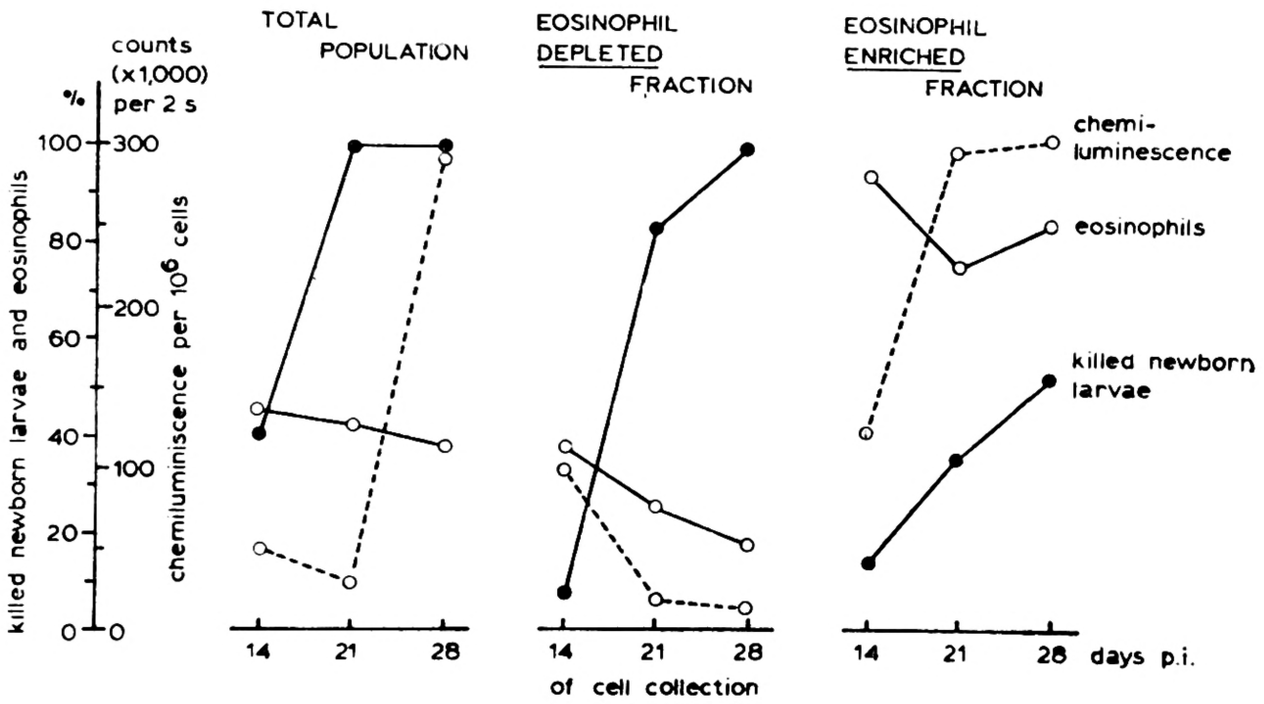

Fig. 1. His dependent larvicidal activity of different cell fractions from the peritoneal cavity of *T. spiralis* infected (day 0 : 5.000 and day 21 : 2.000 larvae) August rats and their capability to show chemiluminescence upon stimulation with PMA (100 ng)

ximally stimulated (with PMA), the eosinophils produced much larger amounts of reactive oxygen metabolites than Mφ's. This difference increased during a *T. spiralis* infection (Figure 1). However, during adherence to the NBL both cell types released low amounts of reactive oxygen metabolites (unpublished results). Various investigators demonstrated that eosinophils during adherence deposited peroxidase positive material on the parasite surface (McLaren et al., 1977). Both observations taken together, i.e. the production of reactive oxygen metabolites and the deposition of peroxidase positive material, made it likely that the oxygen metabolism took part in the cell's killing mechanism. During adherence the cell respires molecular oxygen (O_2) at an increased rate. The membrane bound NADPH-oxidase is activated and reduces the O_2 to superoxide anion (O^-_2) by the transfer of an electron. In this reaction the NADPH is oxidized to $NADP^+$ and a proton is released in the environment i.e. between cell and parasite surfaces. The O^-_2 is than transported to the lysosomes, where in a cascade of enzymic driven reactions the O^-_2 is converted into singlet oxygen ($^1O_2$), hydroxyl radicals ($OH\cdot$) and hydrogen peroxide (H_2O_2) (Sbarra et al., 1978). The lysosomes contain a.o. lysosomal peroxidase, i.e. eosinophil peroxidase (EPO) in eo's (Wever et al., 1981) and myeloperoxidase (MPO) in PMN's and to a lesser extent in young Mφ's.

The lysosomal peroxidases possess binding places for two substrates, H_2O_2 and chloride ions (Cl^-) and catalyzes the formation of hypochlorite (OCl^-) $\cdot$ OCl^- is a highly reactive oxidizing product. The target groups are the amino (NH_2) groups of peptides, double bonded sulfur (S=S) and

double bonds in lipids (C=C) (Thomas, 1979; Wever et al., 1981). It was supposed that OCl^- might react with the aminogroups of cuticle-bound proteins of the NBL. An assay system was introduced in which purified human EPO and MPO were used as enzymes, H_2O_2 and Cl^- as substrates and NBL as targets. The appropriate conditions with respect to pH and ratio Cl^-/H_2O_2 were observed. Appropriate controls were included. It was found that OCl^- kills the NBL within 20 minutes (Table 1). In a kinetic study it was determined that the rate at which MPO catalyzes

TABLE 1

Larvicidal effect of either purified human EPO or MPO on *T. spiralis* newborn larvae in vitro in the enzyme $|H_2O_2|Cl^-$ system. Ratio $Cl^-|H_2O_2 = 10.000$. [EPO] or [MPO] = 80 nM

System	EOSINOPHIL PEROXIDASE % killed pH 5.5 mean ±S.D.	(*n*)	MYELOPEROXIDASE % killed pH 6.16 mean ±S.D.	(*n*)
Enz. $+H_2O_2+Cl^-$	99.5±1	(4)	100	(3)
Controls				
Enz. deleted	4,0±2.5	(3)	3.1±3.2	(3)
H_2O_2 deleted	4.0±6.5	(3)	0.5±0.8	(3)
SO_4^{2-} substituted for Cl^-	3.0±4.3	(4)	0	(3)
Enz.$+H_2O_2+Cl^-$+BSA 0.4%	7.0±2.4	(3)	1.4	(1)
Enz.$+H_2O_2+Cl^-$+Arginine 4 mM	2.0±2.0	(3)	not done	
Enz.$+H_2O_2+Cl^-+NaN_3$ 1 mM	0	(3)	2.0	(1)
Enz.$+H_2O_2+Cl^-$+Catalase 1800 U	0	(2)	0	(2)
NAOCl 16 μM (5×)	98.5±3	(4)	93±11	(4)

n — number of experiments

the formation of OCl^- (Figure 2) is ten times as high as that of EPO (Figure 3). Less than half the amount of OCl^- is needed to kill $\geqslant 50\%$ of the NBL in the MPO than in the EPO containing halogenation. This may explain why MPO-containing cells score higher in NBL killing than eosinophils (Buys et al., 1984). Halogenation of AW and ML was ineffective. These observations have to be repeated in a cell containing assay. However various problems arise. There are no peroxidase specific inhibitors (NaN_3 inhibits also catalase); in Cl^- free systems the cells degenerate; reactions take place in the microenvironment between cell and parasite from which OCl^- scavengers are excluded.

In the cell free halogenation system purified eosinophil MBP was assayed for its scavenging properties. MBP is an arginin rich protein and is, because of its many NH_2 groups, a potential OCl^- scavenger. This supposition was confirmed. The concentration at which MBP prevented the killing of NBL by OCl^- was lower than the concentration at which MBP showed to be toxic. The less efficient killing of NBL by eosino-

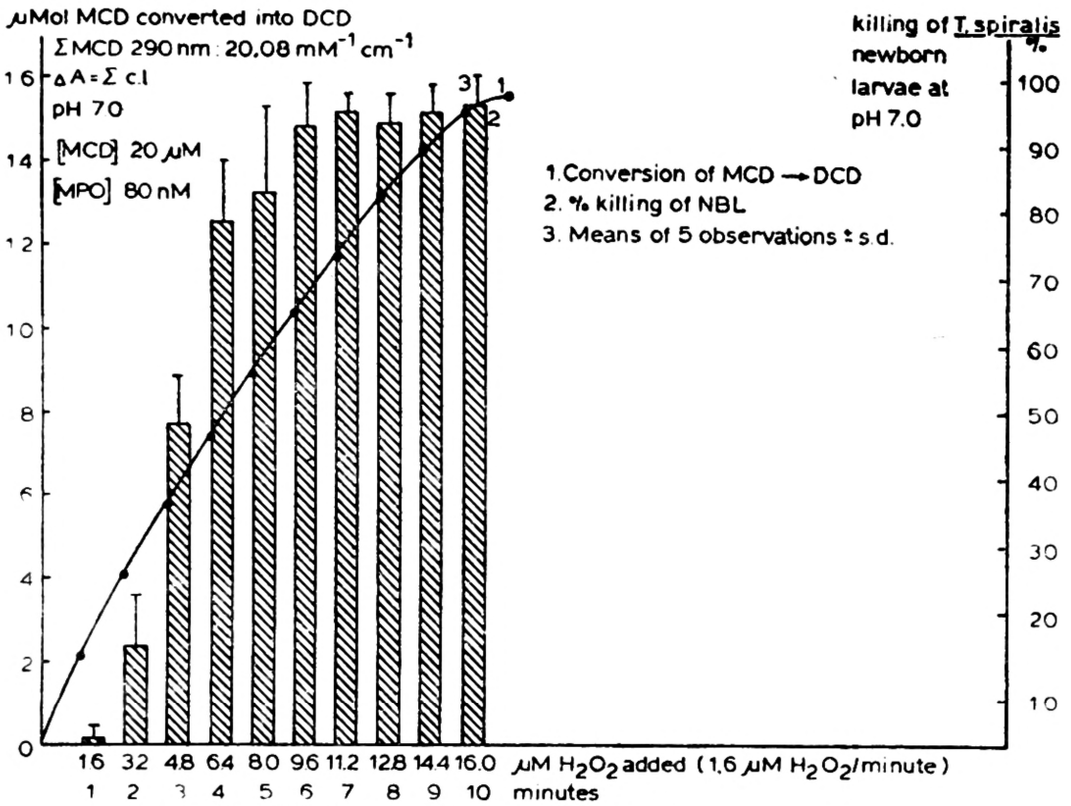

Fig. 2. Killing efficiency of Myeloperoxidase

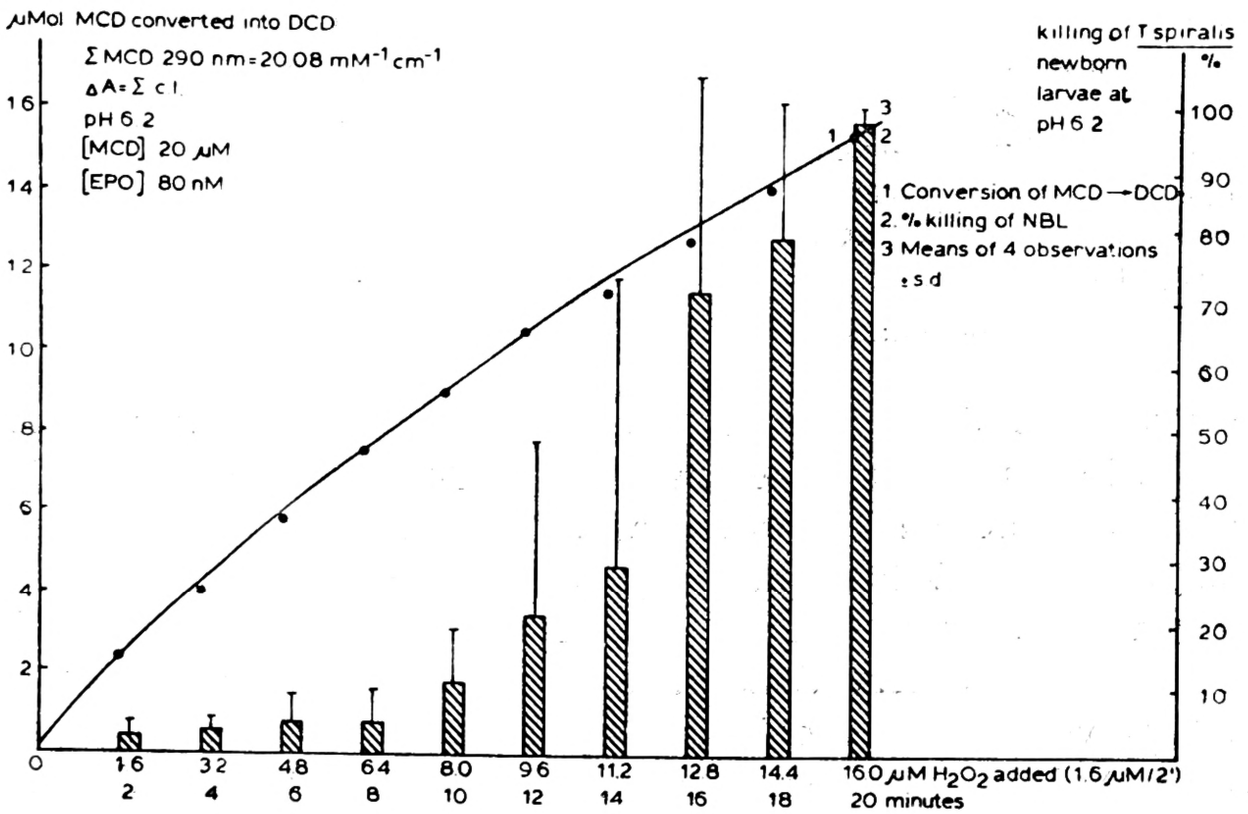

Fig. 3. Killing efficiency of Eosinophil peroxidase

phils as compared to PMN's and M's might be due to the fact that the MBP present in the microenvironment between cell and parasite, served on the one hand as a larvicide, on the other hand, however, as an OCl^- scavenger. As the larvicidal effect of MBP was inferior to that of OCl^-

together with the observation that eosinophils were slow OCl^- producers the net result of NBL killing was likely to be lower than that of PMN's and Mφ's (Buys at al., 1984).

Introduction of biological response modifiers

Biological response modifiers (BRM) are defined as immunostimulants which activate the inflammatory component of the host's defence in an aspecific manner causing the desintegration of pathogens, thus liberating "hidden" antigens which, after being presented to the immune system, induce specific immunity.

Parasites often escape the host defence. Different reasons may form the basis of this phenomenon, i.e. weak immunogenicity, taking up host components and induction of immune suppression. This may mean that parasites after their maturation do not trigger the immune system any more. Once a parasitic infection has established it may remain for months and even years.

The observation that Bacillus Calmette Guérin (BCG) administered i.v. to mice increased the cytotoxicity of peritoneal Mφ's (PMφ's) towards different parasites in vitro opened new perspectives (Cox, 1982). PM's from mice pretreated with BCG 10 days prior to collection showed in the ADCC assay an increase in *T. spiralis* NBL killing from 20% (untreated) to 93% (Perrudet et al., 1985). Similar results were obtained using other BRM's from bacterial origin, muramyldipeptide (a synthetic analogue of *Mycobacterium bovis* cell wall preparation) and the pharmaceutical Levamisole. Susceptible targets included helminths and protozoans (Cox, 1982).

From previous work done in the field of tumour research it was learned that systemically administered BCG did not induce (lasting) tumour regression although PMφ's showed in vitro increased cytotoxicity towards tumour cells. However, when BCG was administered peri- or intratumourally lasting tumour regression occurred. Observance of the appropriate moment of BCG administration proved to be essential (Zbar and Rapp, 1974; Hanna and Peters, 1975). Transfer of the spleen cells from animals thus cured to syngeneic animals protected the latter from tumour development after a challenge inoculation. This protection was tumour specific.

The following mode of action was suggested: BCG administered intratumoural activates the Mφ's already present in the tumour. These Mφ's release i) lysosomal contents among which reactive oxygen metabolites which desintegrate nearby tumour cells; ii) IL-1 which activates T-helper cells and resting B-cells; iii) chemoattractants causing the influx of more

inflammatory cells. T-helper cells produce IL-2 and IF-γ which activate various T-cell subpopulations and APC's respectively (de Jong, 1985) (discussed under 4). The tumour antigens are taken up by APC's for presentation to the immune system and induce antigen receptors on the appropriate cell populations (discussed under 4). Thus, inflammatory and immune operational cells are mobilized and specific immunity is established through an antigenically unrelated stimulant of inflammation. It should be mentioned that not all small laboratory rodents are susceptible to BCG treatment.

Systemic treatment with BCG with regard to parasitic infections showed that the effect was partial and transient in the case of *S. mansoni* and effective but also transient in the case of *Babesia* and *Plasmodium* (Mahmoud, 1980; Clark et al., 1976). However, BCG treatment was given at a well-timed moment prior to infection which can only be applied to experimental investigations. Local treatment of an established parasitic infection is excluded as tissue parasites are scattered over the body and often occupy locations in vital organs. The induction of multiple foci of inflammation as a result of "successful" BCG treatment is unacceptable. However the adjuvant effect of a number of BRM's cannot be denied and may boost specific immunity also where parasites are involved. Not all BRM's are suitable for human use. However, local treatment of patients with tumours of the head and neck region with a BCG cell wall preparation was remarkably successful (Bier et al., 1984). Recently it was found that partial protection was obtained after immunization of mice with an excretory antigen of *Leishmania tropica* crosslinked to muramyldipeptide (Steinberger et al., 1984). These results give hope for future success in finding immunisation procedures with lasting effects against parasitic diseases. Therefore, investigations into the use of BRM's or their derivates in combination with or linked to the appropriate parasitic immunogens may provide an essential constituent of future parasitic vaccines.

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