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The role of cytokines in immunity and immunopathogenesis of pirolasmoses

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Abstract Cells involved in innate and adapted immunity produce cytokines capable of orchestrating the immune response to *Babesia* and *Theileria* infections. Thus, CD4-postive T cells recognise peptide fragments of the parasites in the context of the major histocompatibility complex (MHC) class II antigen and produce gamma interferon (IFN-γ) to activate macrophages for enhanced phagocytosis and intracellular killing of the parasites. In addition, CD4-positive T cells produce interleukin 2 (IL-2) which is essential for the clonal expansion of CD8-positive T cells. The latter cells kill *Theileria*-infected host leucocytes in an MHC class I-dependent manner. On the other hand, the overproduction of proinflammatory cytokines contributes to disease progress.

Introduction

There is increasing evidence that protective immunity to ticks and tick-borne diseases (TBDs) depends on the mobilisation of both innate and adaptive immunity. In this context, cytokines play a crucial role. However, cytokines are not only involved in controlling the infection but also in the exploitation of disease progress.

It is not the aim of this mini-review to analyse the function of cytokines in the whole complex of TBDs, but rather to focus on cytokines in piroplasmoses.

Theileriosis

Cattle which recover from a primary infection are strongly immune when challenged with a homologous parasite stock and, to a certain degree, against some heterologous stocks as well (Mehlhorn et al. 1994). The immunological response of the host is directed against all parasite stages: sporozoites, macroschizonts and merozoites. *Theileria*-specific neutralising antibodies and cytotoxic T lymphocytes (CTL) directed against schizont-infected cells have been demonstrated in both *Theileria parva* and *Theileria annulata* infections (Ahmed and Mehlhorn 1999). Besides the parasite-specific CTLs, another subset of T cells has been detected in cattle immunised against tropical theileriosis or East coast fever, either by infection and treatment or by the inoculation of macroschizont-infected cells. When incubated with autologous parasitised cells, these T cells proliferate and produce interleukin 2 (IL-2) and gamma interferon (IFN-γ) (Ahmed et al. 1999). They have been characterised as $CD3^+$ $CD4^+$ cells expressing high levels of IL-2 receptors (IL-2R), MHC class II antigen and CD45RB (Busseler et al. 1997). Macroschizont-containing cells can also induce naive T cells to proliferate and to secrete Th1 cytokines (Campbell et al. 1995), which might be mediated by superantigens in a manner similar to many other bacterial or parasitic infections.

Neither IFN-γ nor IL-2 has ever been shown to inhibit the growth of or to exert a direct lytic effect on the parasitised cells. However, there is good evidence to indicate that a sub-population of peripheral blood mononuclear cells of infected cattle exhibit a cytostatic effect on the growth of the parasitised cells (Preston and Brown 1988). It has been speculated that IFN-γ activates monocytes/macrophages to produce monokines which may inhibit the growth of and kill parasitised host cells. Later investigations showed that a number of the cytokines tested were unable to interfere with the growth of the infected cells. Nevertheless, the majority of these cytokines prevented the establishment of a new infection by inhibiting the differentiation of the trophozoites of *T. annulata* to schizonts. Most interestingly, nitric oxide (NO) could even kill parasite-containing cells. Besides its protective role, IFN-γ may actively stimulate the growth of parasitised cells (Preston et al. 1999).

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The pathogenesis of theileriosis, caused by *T. parva*, *T. annulata* and *Theileria lestoquardi,* is primarly due to infection of the host's leucocytes by macroschizonts, which proliferate synchronously with their host cells. *T. parva* preferentially infects T cells, whereas *T. annulata* and *T. lestoquardi* appear to transform mainly MHC class II-positive cells (Ahmed et al. 1999; Preston et al. 1999), among which macrophages and B lymphocytes have been identified as targets of *T. annulata*. Interestingly, the inoculation of cattle with infected B cells causes a mild and self-limiting infection, whereas parasitised T cells induce a more severe infection. This does not appear to be cytokine associated as multplex PCR analysis of cytokine expression by a number of bulk and cloned infected cells did not identify any line-specific cytokine profiles (McKeever et al. 1999). In contrast, cytokines produced by *T. annulata*-infected cells or by uninfected responding cells seem to play a role in the pathogenesis of tropical theileriosis. Growth factors like IL-2 enhance the proliferation of parasitised cells by binding to IL-2R which are expressed on the parasitised cells (Ahmed et al. 1999).

Despite a significant expression of IFN-γ, naive cattle are not protected against a severe primary infection with *T. annulata*. Elevated amounts of IFN-γ seem to enhance the growth of the parasitised cells (Campbell et al. 1995). In spite of its expression at the mRNA level, tumour necrosis factor alpha (TNF-α) could not be detected in supernatants of *T. annulata*-infected cell lines (Ahmed et al. 1999). However, taking into consideration that this cytokine is a potent inducer of fever and may play a role in anaemia, muscle wasting and necrosis – symptoms which are also observed in tropical theileriosis – it is reasonable to suggest that TNF-α is involved in the pathogenesis of the disease.

Taken together, the nature of the cells, the cytokines that they produce and their capacity to induce a non-specific activation of naive T cells to proliferate account for the pathogenesis of tropical theileriosis. The role of natural killer (NK) cells is to be determined and has not clearly been associated with the pathogenesis of the disease as has been suggested for *T. parva*.

Babesiosis

Immunity to the protozoan parasite *Babesia* is mediated by both innate and adaptive immune mechanisms. The natural immunity includes the participation of macrophages and NK cells. The later produce the IFN-γ required for the activation of macrophages, which consequently exhibit an enhanced receptor expression, phagocytic activity and enhanced production of toxically acting mediators such as NO. Unlike *Theileria*, *Babesia* parasites reside only within the erythrocytes of their vertebrate hosts. As erythrocytes do not possess major histocompatibility complex (MHC) antigen-presenting cells, such as macrophages, take up the infected erythrocytes and present parasite-derived peptides to $CD4^+$ T cells in the context of MHC class II antigen. Consequently, $CD4^+$ T cells produce IFN-γ which plays a crucial role in activating monocytes/macrophages for an enhanced phagocytosis and NO production. There is strong evidence for a positive correlation between the production of IFN-γ and IgG2a on the one hand and the control of *Babesia* infection on the other (Brown et al. 1998). Supernatants of *Babesia*-specific T-cell lines are able to activate macrophages for enhanced NO production (Stich et al. 1998). Further analysis indicated that these supernatants contain both IFN-γ and TNF-α. Evidence for the toxic effect of NO has been indirectly presented by showing that chemical donors of NO inhibit the in vitro growth of *B. bovis*. Moreover, IFN-γ is involved in the production of opsonising IgG2 antibodies. However, the outcome of the infection in a naive animal is related to the timing and the quantity of the cytokines produced. Thus, the overproduction of pro-inflammatory cytokines contributes to disease progress, leading to cerebral babesiosis and adult respiratory distress syndrome, which often leads to death (Brown and Palmer 1999).

Taken together, the effective control of babesiosis depends on both innate and acquired immunity with a well-balanced cytokine production. In this context, research is directed toward the identification of protective parasite proteins that induce IFN-γ and Ig2 antibody responses. Of great interest will be those parasite components that activate the innate immune responses.

References

Ahmed JS, Mehlhorn H (1999) The cellular basis of the immunity to and immunopathogenesis of tropical theileriosis. Parasitol Res 85:539–549

Ahmed JS, Schnittger L, Mehlhorn H (1999) *Theileria* schizonts induce fundamental alterations in their host cells. Parasitol Res 85:527-538

Brown WC, Palmer GD (1999) Designing blood-stage vaccines against *Babesia bovis* and *Babesia bigemina*. Parasitol Today 10:275–281

Brown WC, McElwain TF, Palmer GH, Chantler SE, Estes DM (1998) Bovine CD4+ positive T-lymphocytes clones specific for rhoptry associated protein-1 (RAP-1) of *Babesia bigemina* stimulate enhanced immunoglobulin G1 (IgG1) and IgG 2 synthesis. Infect Immun 67:155–164

Busseler H, Conze G, Hartwig H, Campbell JDM, Spooner R, Ahmed JS (1997) Characterization of bovine T-cells responding to stimulation with autologous *T. annaulata*-infected cells. Immunobiology 197:358–359

Campbell JDM, Howie SEM, Odling KA, Glass EJ (1995) *Theileria annulata* induces aberrant T-cell activation in vitro and in vivo. Clin Exp Immunol 99:463–470

McKeever DJ, Taracha ELN, Morrison WI, Musoke AJ, Morzaria SP (1999) Protective immune mechanisms against *Theileria parva*: Evolution of vaccine development strategies. Parasitol Today 10:263–267

Mehlhorn H, Schein E, Ahmed JS (1994) *Theileria*. In: Kreier J (ed) Parasitic protozoa. Academic Press, San Diego, pp 217–304

Preston PM, Brown CGD (1988) Macrophage mediated cytostasis and lymphocyte cytotoxicity in cattle immunised with *Theileria annulata* sporozoites or macroschizont-infected cell line. Parasite Immunol 10:531–647

Preston PM, Hall RF, Glass EJ, Campbell JDM, Darghouth MA, Ahmed JS, Shiels BR, SpoonerRL, Jongejan F, Brown CGD (1999) Innate and adaptive immune responses co-operate to protect cattle against *Theileria annulata*. Parasitol Today 15:268–274

Stich RW, Shoda LKM, Dreews M, Adler B, Jungi TW, Brown WC (1998) Stimulation of nitric oxide production in macrophages by *Babesia bovis*. Infect Immun 66:4130–4136