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GRANULOPOIESIS DURING ACUTE *Toxoplasma gondii* INFECTION IN MICE

GORDANA JOVČIĆ, MARIJANA PETAKOV, DIANA BUGARSKI, OLGICA ĐURKOVIĆ-ĐAKOVIĆ, ANA KRAGUJEVIĆ and NEVENKA STOJANOVIĆ

Institute for Medical Research, Dr. Subotića 4, P.O. Box 721, 11001 Beograd, Yugoslavia

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There is little evidence for the role of granulocytes in the host defence against the protozoan parasite Toxoplasma gondii. Thus, the purpose of this study was to assess the involvement of granulocytes during acute murine infection with the parasite virulent RH strain. Since, all infected animals succumb to infection in a dose-dependent period of time, the effect of two parasite doses, $2x10^2$ and $2x10^6$, on granulocytis cell compartments in the bone marrow and peripheral blood up to 2 or 6 days post infection, respectively, was investigated. The data obtained revealed that granulocytic cells at various stages of differentiation and maturation were affected by T.gondii infection regardless of the dose applied. The observed oscillating changes in the number of cells in granulocytic proliferative and nonproliferative compartments imply that parallel with T.gondii-induced damage to granulocytic cells, a process of stimulated production of granulocytes occurred. Although some differences in the kinetics of cellular changes and in the extent of cell damage were found, similar reactivity patterns of granulocytic cells were seen following infection with both doses. Our results demonstrated significant changes in granulopoiesis during T.gondii infection, suggesting a possible contribution of granulocytes to parasite control.

Key words: Toxoplasma gondii, acute infection, mice, granulocytes

INTRODUCTION

Toxoplasma gondii is an ubiquitous protozoan parasite highly infective for most animals and humans. As an obligatory intracellular parasite, *T.gondii* is able to infect and propagate in virtually all nucleated host cells. In healthy subjects the acute infection is generally mild and asymptomatic, due to a potent immune response involving both non-specific and specific mechanism, while parasite latency in the chronic stage of infection is maintained by an adaptive Tcell

response (Kasper & Boothroyd,1993) Severe or life-threatening infections are most commonly recognized in immunocompromised patients, such as patients with AIDS and malignancies, transplant recipients and in congenitally infected infants (Luft & Remington, 1994). The increased incidence of diseases accomparied with suppression of the host immune response has necessitated current investigations concerning the pathogenesis of toxoplasmosis which is still poorly understood.

Since *T.gondii* infection can be easily obtained in most laboratory animals, animal models of toxoplasmosis have been extensively used to study the pathology of the infection. Although data obtained experimentally greatly improved our understanding of various aspects of toxoplasmosis, the effects of *T.gondii* on the hematopoietic system are not yet clear. It is well known that granulocytes are the first cells that migrate into tissues in response to invading pathogens, but there is little evidence for the role of granulocytes in resistance against *T.gondii.* To assess the contribution of granulocytes in host defense we investigated the disease-related changes in the granulocytic cell lineage during acute murine infection with the virulent RH strain of *T.gondii.* Infection with the RH strain leads to generalized pathological changes and ultimately to a lethal outcome and since the survival time post infection is a function of the inoculum size (Djurković-Đaković et al.,1996), the effect of parasite dose on granulopoiesis was studied.

MATERIAL AND METHODS

Animals. The influence of acute toxoplasmosis on granulopoiesis was studied in inbred male CBA mice weighing 20-22 g. Swiss albino mice were used for maintenance of *T.gondii* RH parasites.

Parasites. Tachyzoites of the virulent RH strain of *T. gondii*, maintained through serial intraperitoneal (i.p.) passages in Swiss albino mice, were used. For infection, tachyzoites were obtained from peritoneal exudates of Swiss albino mice infected three days earlier. Tachyzoites were harvested by aspiration, washed several times and the numbers of parasites were adjusted with saline to $4x10^2$/ml or $4x10^6$/ml prior to inoculation.For experimental procedures 0.5 ml of these suspensions were i.p. inoculated into mice.

Experimental protocol. To determine the effect of parasite dose on granulocytic cell lineage CBA mice were injected i.p. with two different doses of RH strain tachyzotes, $2x10^2$ or $2x10^6$. Animals were sacrificed at various time points (3,6, 12, 24, 48, 96 and 144 hours) post infection (p. i.). Normal, noninfected mice were used as controls. In each animal the following hematological parameters were estimated: in the femoral bone marrow, the number of granulocyte-macrophage committed stem cells (CFU-GM), the total number of nucleated cells and the differential count of morphologically recognizable hematopoietic cells; and in peripheral blood, the total number of white blood cell (WBC) and the differential count of nucleated cells. The experiments were conducted twice and 6 to 10 mice were used per group for each time point.

*GFU-GM colony assay.*The number of CFU-GM derived colonies was determined using a methylcellulose cell-culture system with murine lungconditioned

medium CSF (Sheridan & Metcalf, 1973; Stojanović et al., 1988). The mixture containing 5 x 10^4 nucleated bone marrow cells was plated in duplicate and incubated at 37^0C in a humidified atmosphere of 5% CO_2 in air. After 7 days of incubation, the number of colonies representing more than 50 cells was counted.

Estimation of morphologically recognizable bone marrow cells. On bone marrow cell smears stained by the May-Grunwald-Giemsa procedure 1000 nucleated cells were differentiated and divided into the following compartments: proliferative granulocytes - PG (myeloblasts, promyelocytes and myelocytes), metamyelocytes, mature granulocytes, monocytes, lymphocytes, erythroblasts, orthochromatic blasts and other cells including nonidentified cells.

Estimation of peripheral blood cells. Differential counts of nucleated cells were made on 100 counted cells on blood smears stained by the May-Grunwald-Giemsa procedure.

Statistics. The data were analysed employing Student's t-test.

RESULTS

To evaluate the involvement of granulocytes in acute murine toxoplasmosis we examined the effect of two different parasite doses, $2x10^2$ and $2x10^6$, on granulocytic cells at various stages of differentiation and maturation. The chan-

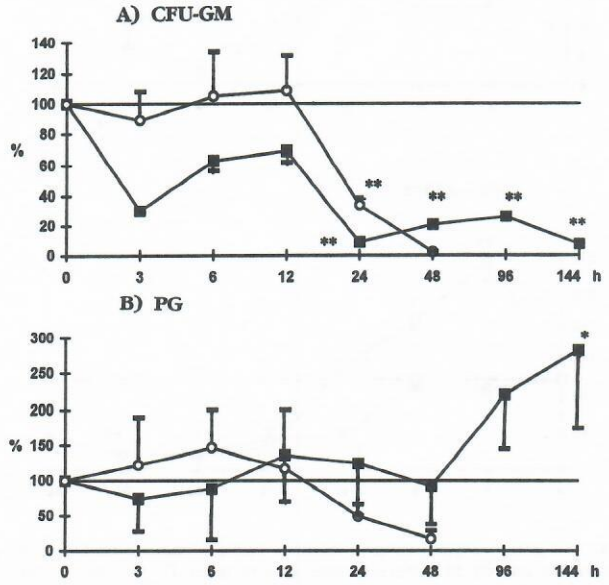

Figure 1. Bone marrow granulocytic proliferative compartments: granulocyte-macrophage committed stem cells - CFU-GM (A) and morphologically recognizable proliferative granulocytes - PG (B) at different time points after *T. gondii* infection presented as a percentage of those in normal, non-infected mice (N=100%) (–) Controls; (-□-) low dose and (-o-) high dose inoculation. Values are the mean æ SD. *p<0.05; **p<0.01.

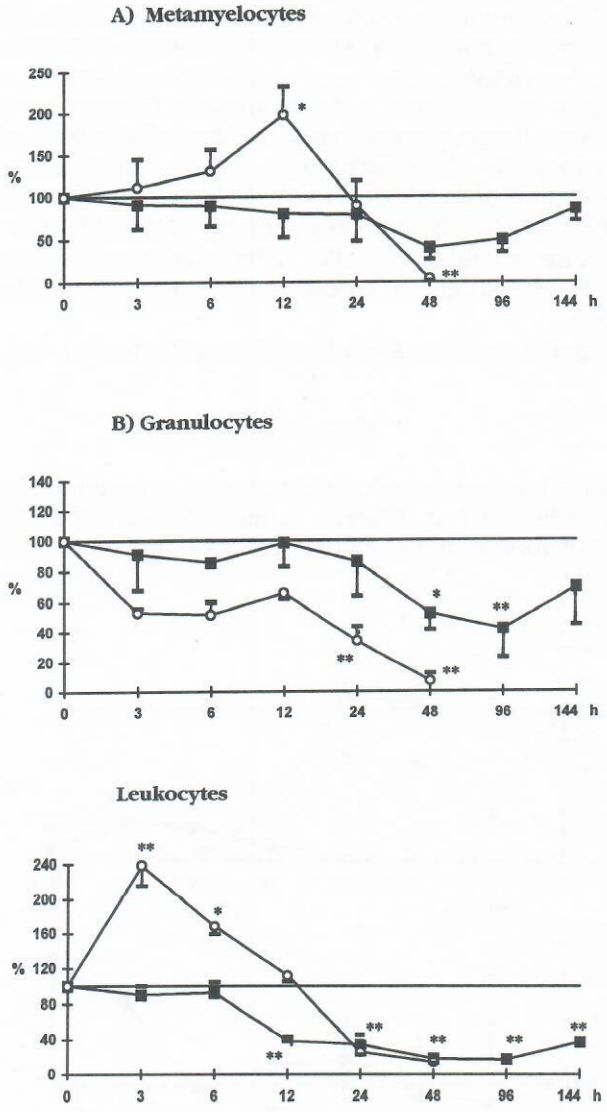

Figure 2. Bone marrow granulocytic nonproliferative compartments: metamyelocytes (A) and mature granulocytes (B) at different time points after *T. gondii* infection presented as a percentage of those in normal, non-infected mice (N=100%). (–) Controls; (-□-) low dose and (-o-) high dose inoculation. Values are the mean ± SD. *p<0.05;**p<0.01.

Figure 3. Peripheral blood leukocytes at different time points after *T. gondii* infection presented as a percentage of those in normal, non-infected mice (N=100% (–) Controls; (-□-) low dose and (-o-) high dose inoculation. Values are the mean ± SD. *p<0.05; **p<0.01.

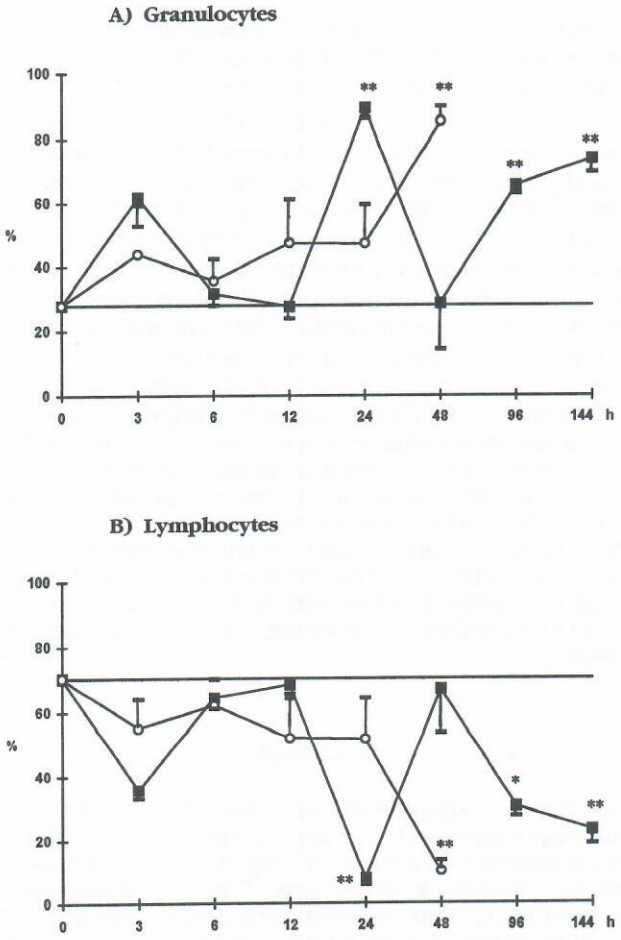

Figure 4. Peripheral blood granulocytes (A) and lymphocytes (B) at different time points after *T.gondii* infection presented as a percentage of those in normal, non-infected mice (N=100%). (–) Controls; (-□-) low dose and (-o-) high dose inoculation. Values are the mean ± SD. *p<0.05; **p<0.01.

ges within granulocytic cell compartments in the bone marrow and peripheral blood were analysed at different time points,up to 2 and 6 days p. i., respectively, since all high dose-infected animals succumbed to the infection within three days, while death after the smaller inoculum occurred between days 7 and 8.

In the bone marrow, estimation of CFU-GM progenitors following *T.gondii* infection showed that both parasite doses basically induced a decline in the number of these cells which was most evident from 24 h p. i. onward, although different reactivity patterns occurred in the first 24 h (Figure 1A). The differential count of morphologically recognizable proliferative granulocytes, demonstrated that during the first 12h p. i. the numbers of these cells in both groups of infected

animals were close to the control values. Afterwards in the lower dose-infected animals the values increased and in the higher dose animals the number of proliiferative granulocytes decreased (Figure 1B).

These changes within femoral granulocytic proliferative compartments pointed to an accelerated flow of CFU-GM through this cell compartment, as well as to an increased proliferation of cells belonging to the morphologically recognizable proliferative granulocytic compartment. The analysis of changes within different nonproliferative granulocytic cell compartments revealed that both parasite doses induced oscillating changes in the number of metamyelocytes (Figure 2A) and a decrease in the number of mature granulocytes (Figure 2B). Taken together, the changes within different femoral granulocytic compartments indicated that parallel with delivery of granulocytes from the bone marrow, stimulation of *de novo* production of granulocytic cells took place.

Analysis of hematological parameters in peripheral blood demonstrated that the lower parasite dose induced a permanent decrease in the total number of white blood cells from 6 h p. i. onward, while the higher dose, following an initial increase, caused a significant reduction in the number of circulating leukocytes (Figure 3). Although the number of leukocytes declined, observed changes in the relative proportion of various white blood cell types exhibited an almost constant pattern. Namely, according to the differential count, the percentage of granulocytes was permanently increased and the percentage of lymphocytes decreased (Figure 4), even though the kinetics of these changes differed depending on the parasite dose.

DISCUSSION

The involvement of granulocytes in inflammatory and immune responses as primarily phagocytic cells and their importance in killing extracellular bacteria has long been established, but there is only indirect evidence for their role in resistance against intracellular organisms. Thus, to investigate the possible contribution of granulocytes to parasite control we have studied the effect of *T.gondii* on granulopoiesis during acute infection in an experimental mouse model. The *T.gondii* RH strain used is known as the most virulent and all animals succumb to infection, even following inoculation of a theoretically single parasite (Djurković-Djaković et al., 1996). Since the length of survival is dose-dependent, two parasite doses, $2x10^2$ or $2x10^6$, were chosen.

The data obtained clearly demonstrated that granulocytic cells at various stages of differentiation and maturation were affected by *T.gondii* infection regardless of the dose applied. The observed changes imply that in spite of the *T.gondii*-induced damage of the granulocyte cell lineage, a process of stimulated production of granulocytes occurred.

In the bone marrow, the time course decrease in the number of femoral mature granulocytes observed in *T.gondii* infected animals suggested either that *T.gondii* induced damage to the granulocytic cells or that the infection induced mobilization of mature cells from the bone marrow. If the damage to the granulocytic cells existed only during the infection, a persistent decline in the number of cells in different granulocytic compartments could be expected. However, the oscillating changes in the number of cells in granulocytic prolifera-

tive and nonproliferative compartments indicated that, in parallel with the delivery from the granulocyte-storage pool, a process of stimulated production of granulocytes occurred. Stimulated bone marrow granulopoiesis was not always manifested by a persistant increase in the number of cells in the granulocytic compartments, but rather with oscillating changes or decreased values. Since each compartment precedes and feeds the next one, oscillating changes and decline in the cell number actually imply to an accelerated flow through the compartment. Namely, the increased cell flow through the granulocytic compartments does not necessarily require an increased cell number of a particular compartment, because the outflow goes up.

In the peripheral blood, *T. gondii* infection caused a significant decrease in the total number of white blood cells. However, differential counts showed significant differences in the granulocyte and lymphocyte composition compared to pre-infection levels. Namely, the relative proportion of granulocytes and lymphocytes was changed in favor of granulocytes, probably as a consequence of changes observed within bone marrow granulocytic cell compartments, suggesting that stimulation of granulopoiesis occurred.

As regards the effect of parasite dose on granulocytic cells, some differences in the kinetics of the cellular changes took place, but the most profound distinction between the two doses was the extent of observed cell damage. In all high dose-infected animals the numbers of cells in all analysed bone marrow granulocytic compartments and peripheral blood leukocytes reached very low levels by the end of the observation period. Meanwhile, the numbers of granulocytic cells in low-dose infected mice, although variable over time, mainly remained within the values found in the control, non-infected animals, or were even increased as in the case of marphologically recognizable proliferative granulocytes. Nevertheless, although differences were found, basically similar reactivity patterns of granulocytic cells were seen in all infected animals confirming the presence of stimulated granulopoiesis. However, during a massive severe infection such as acute toxoplasmosis induced by RH strain, the effect of granulocytic activation could not alter the obligatory lethal outcome of the disease.

The results obtained in these experiments show the significance of the granulocyte contribution during *T. gondii* infection. Previously, it was reported that human circulating phagocytes, both monocytes and polymorphonuclear leukocytes, kill intracellular *T. gondii* tachyzoites *in vitro* (Wilson & Remington, 1979). Recently, neutrophils have been shown to play an important role in innate resistance to *L. monocytogenes* (Rogers & Unanue, 1993) and *Candida albicans* (Romani et al., 1996) in murine models. Moreover, it was pointed out that neutrophils appear to contribute to acute resistance to *T.gondii* (Scharton-Kersten et al., 1997). Further studies are needed to evaluate the extent and importance of granulocytic participation in the organism's response to *T.gondii* infection, since in the past few years, these cells are recognized not only as cells engaged in phagocytosis, but also as cells that have the ability to synthesize and relase immunoregulatory cytokines and, through cytokine secretion, they may influence the direction and evolution of immune processes (Cassatella, 1995).

A c k n o w l e d g m e n t

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GRANULOPOEZA U AKUTNOJ EKSPERIMENTALNOJ TOKSOPLAZMOZI

GORDANA JOVČIĆ, MARIJANA PETAKOV, DIANA BUGARSKI, OLGICA DJURKOVIĆ-DJAKOVIĆ ANA KRAGUJEVIĆ i NEVENKA STOJANOVIĆ

SADRŽAJ

S obzirom da ima vrlo malo podataka o ulozi granulocita u odbrani organizma od infekcije izazvane parazitom *Toxopasma gondii,* u ovom radu na eksperimentalnom modelu miša sa akutnom toksoplazmom izazvanom visoko virulentnim RH sojem parazita ispitivan je uticaj infekcije na ćelije granulocitne loze. Kako je dužina preživljavanja životinja nakon inokulacije parazita direktno zavisna od primenjene doze praćen je efekat dve doze parazita, $2x10^2$ i $2x10^6$, na različite kategorije ćelija granulocitne loze. Dobijeni rezultati su pokazali da infekcija sa *T.gondii* uzrokuje promene u okviru svih kategorija ćelija granulocitne loze, nezavisno od stepena njihove diferentovanosti i zrelosti. Ove promene, i to kako u okviru proliferativnih granulocita, tako i neproliferativnih granulocita u kostnoj srži i perifernoj krvi, ukazale su da paralelno sa destrukcijom ovih ćelija izazvanom *T. gondii*, dolazi i do njihovog povećanog stvaranja. Iako su uočene neke razlike u vremenskoj kinetici i intenzitetu promena, sličan odgovor ćelija granulocitne loze zapažen je bez obzira na visinu doze infekcije. Rezultati naših istraživanja pokazali su da tokom infekcije dolazi do značajnih promena u okviru procesa granulopoeze, što ukazuje na mogući doprinos granulocita u imunskom i protektivnom odgovoru na *T.gondii.*