NOTE

Parasitology

Cytokine mRNA Profiles in Bovine Macrophages Stimulated with Trypanosoma congolense

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(Received 25 July 2006/Accepted 12 December 2006)

ABSTRACT. It is known that different breeds of cattle display differential susceptibilities to Trypanosoma congolense infections, and that N'Dama cattle remain more productive after infection than Boran cattle which are more susceptible to T. congolense. Macrophages from both breeds were cultured in vitro and the expressions of a number of cytokines and iNOS mRNA were analyzed using real time RT-PCR after stimulation with antibody-opsonized trypanosomes. No significant difference was seen between the responses of the two breeds. However, RNA levels of TNF-α in the IFN-γ-primed macrophages were about 100-fold higher than those in the non-primed macrophages. A significant ten-fold decrease was seen for the anti-inflammatory cytokine IL-10. These results indicate that priming of the cells with IFN-γ cause a serious shift toward an inflammatory response.

KEY WORDS: cytokine, macrophage, Trypanosoma congolense.

African trypanosomes are extracellular parasites that cause sleeping sickness in humans and nagana disease in livestock in sub-Saharan Africa. Different breeds of cattle are known to display differential susceptibilities to trypanosome infections, and N’Dama cattle remain more productive after infection than Boran cattle which are more susceptible to trypanosomes. The mechanisms in N’Dama cattle to bring about this trypanotolerance are unknown, but they impart stronger abilities to control parasite growth and to limit the associated anemia. The mechanism to limit anemia seems to be the most important in maintaining productivity. Although N’Dama cattle develop anemia, it dose not reach the degree of severity as that seen in susceptible Boran cattle, and will be recovered faster [7].

Different inbred mouse strains also show different resistance to trypanosomal infection. BALB/c mice are susceptible, while C57BI/6 mice are relatively resistant. Several reports have described the differences in the cytokine levels of macrophages from the resistant and susceptible inbred mice; the macrophages from susceptible BALB/c mice produced more IL-10 and IL-6 than those from resistant C57BI/6 mice, and TNF-α and IL-12 production abilities of macrophages from C57BI/6 were higher than those from BALB/c mice [4]. The macrophages from C57BI/6 mice have been reported to produce more nitric oxide (NO) than the BALB/c macrophages in response to T. congolense [9].

However, these phenomena could not be confirmed in T. congolense-infected cattle as no increases were recognized in the levels of NO and TNF-α mRNA in PBMC from the infected calves [10].

In the present study, we purified macrophages from Boran and N’Dama cattle, and investigated the cytokine mRNA profiles of these macrophages after stimulation with T. congolense.

Peripheral blood monocytes of N’Dama and Boran cattle were collected by means of a magnetic cell separation system (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany). Briefly, venous blood was diluted with an equal volume of Alsever’s solution, layered over Ficoll-Paque Plus (Amersham Pharmacia Biotec AB, Uppsala, Sweden), and centrifuged at 400 × g for 30 min at 20°C. The peripheral blood mononuclear cells (PBMC) band was removed and cells were washed twice with PBS. PBMC were suspended in RPMI 1640 medium containing 5% heat-inactivated fetal calf serum and 100 μg/ml antibiotics (penicillin and streptomycin). PBMC were incubated with anti-human CD14 antibody (Miltenyi Biotec) according to the manufacturer’s instructions.

For preparation of monocyte-derived macrophages, CD14-positive monocytes were cultured in RPMI1640 plus 10% heat-inactivate fetal calf serum and 100 μg/ml of recombinant bovine M-CSF [11] at 5 × 105 cells per well of a 24-well plate for 6 days. Half of the wells were treated with recombinant bovine IFN-γ (10 ng/ml, CIBA-GEIGY, Ltd., Basel, Switzerland) for 24 hr after five days of cultivation.

Ten million in vitro cultured T. congolense (clone IL-1180) were opsonized with 1 ml of bovine serum purified from cattle experimentally infected with the same T. congolense clone for 1 min on ice. After washing with PBS at 1,600 × g for 10 min, they were added to the macrophages at 5 × 106 per well. RNAs were extracted 3 and 6 hr later using TRIzol reagent (Invitrogen, Carlsbad, CA). After treatment with 1 unit of DNase I (Invitrogen), 25 μg of respective total RNA was provided for real time RT-PCR (Applied Biosystem 7500, Applied Biosystems, Foster city, CA) using SYBR Green RT-PCR Reagents (Applied Biosystems). The sequences of the PCR primers for the cytokines were quoted from published papers (IL-1α and β, IL-6, IL-10, IL-12p40 and β-actin, [5], TNF-α; [1]). The PCR primer
Fig. 1. Cytokine mRNA expression profiles in the macrophages stimulated with trypanosome. Circles and full lines: macrophages from N’Dama without IFN-γ treatment. Circles and dotted lines: macrophages from N’Dama with IFN-γ treatment. Triangles and full lines: macrophages from Boran without IFN-γ treatment. Triangles and dotted lines: macrophages from Boran with IFN-γ treatment. *: p<0.05, Boran’s macrophages between with and without IFN-γ stimulation. **: p<0.05, N’Dama’s macrophages between with and without IFN-γ stimulation.
sequences for iNOS were designed based on the sequence of the bovine iNOS gene from the EMBL/Gen Bank databases, AF340231 (sense: 5’-GGCTACGGAACTGGACATCAA C-3’, anti-sense: 5’-CTCAGGGATTCTGGAGTCCTT-3”).

Preliminary experiments were carried out to establish the optimum conditions for the induction of cytokine mRNA responses. The antibody-opsonized trypanosomes induced more cytokine mRNAs than the trypanosomes without opsonization (data not shown).

Figure 1 compares the mRNA responses of a series of cytokines in the macrophages from Boran and N’Dama cattle stimulated by opsonized trypanosomes. No significant differences were seen between the responses of the 2 breeds. The responses from the macrophages that were primed with IFN-γ for 24 hr reacted much more significantly by trypanosome stimulation. Especially, RNA levels of TNF-α in the IFN-γ-primed macrophages were about 100-fold higher than those in the non-primed cells. The priming also caused significant increases in inflammatory cytokines IL-1α, IL-1β, IL-12p40 and iNOS which increased about ten-fold compared to non-primed cells. IL-10 has been shown to suppress the production of inflammatory cytokines [6], and exogenous IFN-γ has inhibited the production of IL-10 by the Staphylococcus aureus Cowan-activated monocytes [2]. In this study, a significant ten-fold decrease was seen for the anti-inflammatory cytokine IL-10.

Administration of anti-IL-10R has been shown to significantly shorten the survival time of mice infected with T. congolense. However, this acute death of T. congolense-infected mice treated with anti-IL-10R was prevented by the administration of anti-IFN-γ antibody [8]. Moreover, IL-10 knockout mice had a lower parasite burden and higher levels of serum TNF-α, IL-12, and IFN-γ compared with normal mice in the case of infection with Trypanosoma cruzi, though the survival time of these knockout mice was shortened [3]. These reports suggest that the balance of production of IFN-γ and IL-10 is important in preventing the development of trypanosomiasis.

In this study, we demonstrated that IFN-γ activated macrophages enhanced the expression of inflammatory cytokine genes such as TNF-α, IL-1α and β, IL-12p40 and iNOS gene. However, these cells decreased expression levels of the IL-10 gene. These results suggest that IFN-γ activated macrophages enlarge the immune responses to reduce the number of parasites by secreting inflammatory cytokines and by reducing expression level of macrophage suppressing cytokine, IL-10. Although the comparison of those cytokine mRNA expressions in the macrophages from both cattle breeds after in vitro stimulation with T. congolense did not show a difference, the priming of macrophages with IFN-γ caused a serious shift toward an inflammatory response. It should be important to compare Boran and N’Dama cattle in terms of the expression levels of IFN-γ and IL-10 in their immune tissues such as liver, spleen and lymph nodes for the next step.

ACKNOWLEDGMENT. This study was conducted under the Collaborative Research Project between JIRCAS and ILRI.

REFERENCES