Alternatively Activated Myeloid Cells Limit Pathogenicity Associated with African Trypanosomiasis through the IL-10 Inducible Gene Selenoprotein P

Tom Bosschaerts, Martin Guilliams, Wim Noel, Michel Hérin, Raymond F. Burk, Kristina E. Hill, Lea Brys, Geert Raes, Gholamreza Hassanzadeh Ghassabeh, Patrick De Baetselier and Alain Beschin

*References*

This article cites 74 articles, 24 of which you can access for free at:
http://www.jimmunol.org/content/180/9/6168.full#ref-list-1

*Subscription*

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

*Permissions*

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

*Email Alerts*

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

*Why The JI?*

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average*
Alternatively Activated Myeloid Cells Limit Pathogenicity Associated with African Trypanosomiasis through the IL-10 Inducible Gene Selenoprotein P

Tom Bosschaerts,*† Martin Guiliams,*† Wim Noel,*† Michel Hérin,‡ Raymond F. Burk,§ Kristina E. Hill,¶ Lea Brys,*† Geert Raes,*† Gholamreza Hassanzadeh Ghassabeh,*† Patrick De Baetselier,*† and Alain Beschin*†‡

Uncontrolled inflammation is a major cause of tissue injury/pathogenicity often resulting in death of a host infected with African trypanosomes. Thus, comparing the immune response in hosts that develop different degrees of disease severity represents a promising approach to discover processes contributing to trypanosomiasis control. It is known that limitation of pathogenicity requires a transition in the course of infection, from an IFN-γ-dependent response resulting in the development of classically activated macrophages (M1), to a counterbalancing IL-10-dependent response associated with alternatively activated macrophages (M2). Herein, mechanisms and downstream effectors by which M2 contribute to lower the pathogenicity and the associated susceptibility to African trypanosomiasis have been explored. Gene expression analysis in IL-10 knockout and wild-type mice, that are susceptible and relatively resistant to Trypanosoma congolense infection, respectively, revealed a number of IL-10-inducible genes expressed by M2, including Sepp1 coding for selenoprotein P. Functional analyses confirm that selenoprotein P contributes to limit disease severity through anti-oxidant activity. Indeed, Sepp1 knockout mice, but not Sepp1 Δ240–361 mice retaining the anti-oxidant motif but lacking the selenium transporter domain of selenoprotein P, exhibited increased tissue injury that associated with increased production of reactive oxygen species and increased apoptosis in the liver immune cells, reduced parasite clearance capacity of myeloid cells, and decreased survival. These data validate M2-associated molecules as functioning in reducing the impact of parasite infection on the host. The Journal of Immunology, 2008, 180: 6168–6175.

The plasticity of macrophages and other CD11b+ myeloid cells (MCs)3 in response to microenvironmental signals results in a continuum of different activation forms. At one end of the spectrum, type 1 cytokine-associated MCs (M1)-induced by IFN-γ, TNF-α, and microbial products like LPS represent the classical form of activation state and play a critical role in type 1 inflammation and in the fight against intracellular pathogens and tumors. At the other end of the spectrum, distinct subsets 4

4Department of Molecular and Cellular Interactions and ¹Laboratory of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Brussel; ²Cell and Tissue Laboratory, Unité de Recherche en Physiologie Moléculaire, Facultés Universitaires Notre-Dame de la Paix, Namur, Belgium; and ³Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37232

Received for publication July 16, 2007. Accepted for publication February 20, 2008.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work, performed in frame of an Interverniversity Attraction Pole Program, was supported by grants from the Institute for Promotion of Innovation by Science and Technology in Flanders and the Fund for Scientific Research Flanders, and by a grant from the Institute for Promotion of Innovation by Science and Technology in Flanders for Generisch Basisonderzoek aan de Universiteiten. R.F.B. and K.E.H. are supported by National Institutes of Health Grant ES02497.

2 Address correspondence and reprint requests to Dr. Alain Beschin, Department of Molecular and Cellular Interactions, Vrije Universiteit Brussel, Laboratory of Cellular and Molecular Immunology, Pleinlaan 2, 1050 Brussels, Belgium. E-mail address: abeschin@vub.ac.be

3 Abbreviations used in this paper: MC, myeloid cell; ROS, reactive oxygen species; Sepp1, selenoprotein P; Sepp1 Δ240–361, selenoprotein P truncated of amino acids 240–361; Ciss, cathespin S; Ngfb, nerve growth factor β; F13a1, coagulation factor XIII, A1 subunit; Treg, T regulatory cell; KO, knockout.

Copyright © 2008 by The American Association of Immunologists, Inc. 0022-1767/08/$2.00

www.jimmunol.org
and IL-12 as well as reactive oxygen and nitrogen species (8–14).

This type 1 immune response is followed at later stage by the emergence of M2 and the expansion of Foxp3+CD4+ regulatory T cells (Tregs) in type 2 cytokine environment in T. congolense- but not in T. brucei-infected C57BL/6 mice. Moreover, the inability of T. brucei-infected mice to suppress the type 1 immune response correlates with the increased pathogenicity of the disease that results in tissue injury and early death of the host (15–18). Of note, in both T. congolense- and T. brucei-infected mice, IL-10 is able to avoid the inflammation mediated by IFN-γ, including overwhelming activation of M1, that results in destruction of the liver and uncontrolled parasite growth (13, 17, 18). Since 80% of these extracellular parasites have been suggested to be cleared from the circulation by liver MCs (19), these data indicate that IL-10, by reducing the pathogenicity of the disease, protects the integrity of the liver and hereby its trypanosome clearance capacity.

Considering the essential role of IL-10 in limiting the pathogenicity and, thus, the susceptibility to the infection, the set of genes induced in M2 of C57BL/6 mice that are relatively resistant to T. congolense infection (2) provides a basis for exploration of the processes that prevent disease severity in African trypanosomiasis. Herein, a number of genes that are inducible by IL-10 in the liver of T. congolense-infected C57BL/6 mice were identified. In addition, we demonstrated that one of these M2-associated genes, Sepp1 which codes for selenoprotein P, is clearly involved in the limitation of the pathogenicity associated with T. congolense infection and, thus, decreases the impact of the inflammatory immune response on the host.

Materials and Methods

Mice, parasites, and infections

Female wild-type and IL-10 knockout (KO) C57BL/6 mice were purchased from Harlan and B&K Universal respectively. Heterozygous Sepp1 KO mice or Sepp1+/- mice that had been backcrossed 10 times with C57BL/6 mice were mated, and female wild-type, Sepp1 KO, and Sepp1+/- mice were fed from the time of weaning Torula yeast-based diet (22) supplemented with 1 mg selenium/kg as sodium selenite (prepared by Harlan Sprague Dawley). Mice were kept under CO2 and livers were perfused through the portal vein with 10 ml Hanks Balanced Salt Solution (HBSS) supplemented with 2 mM EDTA. Then, the liver was minced and isolated (CO2) and livers were perfused through the portal vein with 10 ml HBSS supplemented with 2 mM collagenase type III (Worthington Biochemical Corporation) and 100 U/ml collagenase type II (Sigma-Aldrich). Mice were euthanized (CO2) and livers were perfused through the portal vein with 10 ml HBSS supplemented with 2 mM EDTA, 10% FCS, and overlaid on 10 ml of Lymphoprep (Lymphoprep Bioproducts). After centrifugation (430 g, 25 min, 17°C), the layer of low-density cells at the interface containing non-parenchymal cells was harvested. MCs were isolated by incubation of non-parenchymal cells with MACS CD11b Microbeads (Miltenyi Biotec; 10 µl/10^6 cells) in MACS buffer (CaCl2/ MgCl2) free HBSS, 2 mM EDTA, and 0.5% BSA) and passage through two consecutive positive LS selection columns (Miltenyi Biotec). The CD11b− cell fraction was collected as flow-through during washing of the columns with ice-cold MACS buffer. Purity of isolated MCs checked by flow cytometry, on FACSCanto II using the FlowJo program, always exceeded 95%.

Cytokines and nitrite determination

Blood collected by heart puncture on heparin (20 U/ml) was centrifuged (10 000 g, 10 min) and stored at −80°C. Cytokines were quantified in the plasma using ELISAs for IFN-γ, IL-10 (Pharmingen), or TNF-α (R&D Systems) according to the manufacturer’s protocols. NO level was determined by quantifying the accumulation of nitrate and nitrite in plasma as in Ref. 13.

Differential gene expression analysis

Gene expression was analyzed by quantitative real time PCR using the conditions and primers described in Ref. 2. Results of the PCR analyses were normalized against the house-keeping gene S12, in at least two independent experiments involving at least five mice per condition.

Reactive oxygen species (ROS)

ROS production was measured using H2DCFDA as a probe. Briefly, cells (10^6) were incubated in serum-free DMEM containing 2 µM H2DCFDA (30 min, 37°C), washed twice with excess cold RPMI 1640, and analyzed by flow cytometry.

Apoptosis

Cells were incubated with FITC-Annexin V (BD Biosciences; 4 µl/10^6 cells, 15 min, room temperature) in binding buffer (10 mM HEPS, 140 mM NaCl, and 2.5 mM CaCl2 (pH 7.4)). After washing with binding buffer, cells were analyzed by flow cytometry.

Flow cytometry

After blocking FcR with 2.4G2 anti-CD16/32 Abs (BD Biosciences), cells were analyzed by flow cytometry.

Statistical analysis

Results are presented as mean ± SEM. All comparisons were tested for statistical significance (p < 0.05) using the unpaired t test from GraphPad Prism 4.0 software. Studies were performed on 3–5 independent experiments.

Results

M2-associated genes

Liver non-parenchymal cells were isolated as follows: animals were euthanized (CO2) and livers were perfused through the portal vein with 10 ml of 100 U/ml collagenase type III (Worthington Biochemical Corporation) in HBSS supplemented with 2 mM EDTA. Then, the liver was minced and incubated in 10 ml of 100 U/ml collagenase III (20 min, 37°C). The resulting cell suspension was passed through a 100 µm nylon mesh filter and then centrifuged (300 g, 10 min, 4°C). After erythrocyte lysis, the pellet was resuspended in 10 ml of HBSS supplemented with 2 mM EDTA, 10% FCS, and overlaid on 10 ml of Lymphoprep (Lymphoprep Bioproducts). After centrifugation (430 g, 25 min, 17°C), the layer of low-density cells at the interface containing non-parenchymal cells was harvested. MCs were isolated by incubation of non-parenchymal cells with MACS CD11b Microbeads (Miltenyi Biotec; 10 µl/10^6 cells) in MACS buffer (CaCl2/MgCl2 free HBSS, 2 mM EDTA, and 0.5% BSA) and passage through two consecutive positive LS selection columns (Miltenyi Biotec). The CD11b− cell fraction was collected as flow-through during washing of the columns with ice-cold MACS buffer. Purity of isolated MCs checked by flow cytometry, on FACSCanto II using the FlowJo program, always exceeded 95%.

Cytokines and nitrite determination

Blood collected by heart puncture on heparin (20 U/ml) was centrifuged (10 000 g, 10 min) and stored at −80°C. Cytokines were quantified in the plasma using ELISAs for IFN-γ, IL-10 (Pharmingen), or TNF-α (R&D Systems) according to the manufacturer’s protocols. NO level was determined by quantifying the accumulation of nitrate and nitrite in plasma as in Ref. 13.

Differential gene expression analysis

Gene expression was analyzed by quantitative real time PCR using the conditions and primers described in Ref. 2. Results of the PCR analyses were normalized against the house-keeping gene S12, in at least two independent experiments involving at least five mice per condition.

Reactive oxygen species (ROS)

ROS production was measured using H2DCFDA as a probe. Briefly, cells (10^6) were incubated in serum-free DMEM containing 2 µM H2DCFDA (30 min, 37°C), washed twice with excess cold RPMI 1640, and analyzed by flow cytometry.

Apoptosis

Cells were incubated with FITC-Annexin V (BD Biosciences; 4 µl/10^6 cells, 15 min, room temperature) in binding buffer (10 mM HEPS, 140 mM NaCl, and 2.5 mM CaCl2 (pH 7.4)). After washing with binding buffer, cells were analyzed by flow cytometry.

Flow cytometry

After blocking FcR with 2.4G2 anti-CD16/32 Abs (BD Biosciences), cells were incubated with PE-anti-CD11b Abs (BD Biosciences; 1 µg/10^6 cells, 30 min, 4°C, in the dark). After washing with ice-cold RPMI 1640, cells were analyzed on FACSVantage station (BD Biosciences) using CellQuest software.

Alanine aminotransferase (ALT) levels

Liver glutamic pyruvic transaminase/alanine aminotransf erase (ALT) levels were measured in individual serum samples, using a commercial kit (Boehringer Mannheim).

Statistical analysis

Results are presented as mean ± SEM. All comparisons were tested for statistical significance (p < 0.05) using the unpaired t test from GraphPad Prism 4.0 software. Studies were performed on 3–5 independent experiments.

Results

1. M2-associated genes

Cts1, F13a1, Sepp1, and Ngfb are up-regulated upon T. congolense infection in C57BL/6 mice but not in relatively susceptible IL-10 KO C57BL/6 mice

To envisage a potential role of M2 in the outcome of African trypanosomiasis, the expression of 30 recently identified M2-associated genes (2) was assessed in CD11b− MC fraction from the liver of T. congolense-infected C57BL/6 mice that gradually expanded in the course of infection (Table I). Genes were considered significantly induced if the expression in CD11b− myeloid cells from all infected mice tested (n ≥ 5) was >3-fold higher than in all noninfected mice (n ≥ 5). Twelve genes were found using real time PCR to be preferentially induced in M2 expanding in the course of the liver of T. congolense-infected C57BL/6 mice (Pla2g7, phospholipase A2, group VII; PsaP, prosaposin; Sepp1, selenoprotein P, plasma; 1; Trem2, triggering receptor expressed on MC 2; Ngfb, nerve growth factor β; F13a1, coagulation factor XIII, A1 subunit; Pmp22, peripheral myelin protein; Chi3l3, ym1/2; Mrc1, mannose receptor C, type 1; Follr2, folate receptor 2 (fetal); Lrg1, leucine-rich α-2-glycoprotein 1; and Cts1, cathepsin S).
A kinetic of expression revealed that most of the above mentioned M2-associated genes were significantly induced from days 21–28 post T. congolense infection (not shown), with the exception of the genes coding for selenoprotein P (Sepp1), nerve growth factor β (Ngbf), cathespin S (Ctss), and coagulation factor XIIIa (F13a1) that appeared significantly induced as soon as the first peak of parasitemia was controlled (day 9 post infection) (Table II). Of note, the induction of the latter four genes coincided with the time at which the IFN-γ titer in the plasma of T. congolense-infected C57BL/6 mice returned to normal values compared to a gradual increase of the IL-10 titer (18, 25). The early induction of expression of these genes coinciding with the time of emergence of IL-10 suggests their IL-10 dependence.

To address the mechanisms by which the subset of M2 triggered in an IL-10 environment limit the pathogenicity and render the host relatively resistant to the disease, the level of expression of the M2-associated genes Ctss, F13a1, Sepp1, and Ngbf was determined in T. congolense-infected IL-10 KO C57BL/6 mice and compared with the level of expression observed in wild-type C57BL/6 mice. This analysis was performed at day 9 post infection considering the short survival of IL-10 KO mice (Table III). The level of expression of all genes tested in infected IL-10 KO mice returned to the level of noninfected wild-type mice. Thus, the induction of Ctss, F13a1, Sepp1, and Ngbf can be considered as IL-10 dependent in T. congolense-infected C57BL/6 animals. This observation is highly suggestive for a role of the respective gene products in the IL-10-dependent control of disease symptoms in African trypanosomiasis.

2. Sepp1 KO mice are more susceptible to T. congolense infection

Sepp1 was selected to test whether the IL-10-induced, M2-associated genes limit the pathogenicity and favor the resistance to T. congolense infection. Selenoprotein P, the gene product of Sepp1, is postulated to be an antioxidant molecule (26–28), and ROS, including peroxynitrite, are formed during African trypanosomiasis (29, 30). However, since ROS also cause liver injury (31), we hypothesized that an increased production of selenoprotein P in liver MCs limits the pathogenicity of African trypanosomiasis by detoxifying ROS.

To test this possibility, the course of T. congolense infection was compared in Sepp1 KO and wild-type C57BL/6 mice. Sepp1 KO mice controlled the first peak of parasitemia less efficiently than did wild-type animals, exhibiting higher parasite burden and impaired parasite clearance capacity (Fig. 1). Approximately 50% of Sepp1 KO mice died around 9 days post infection, i.e., 3 days after the first peak of parasitemia was reached. Yet, Sepp1 KO mice that overcame the early wave of parasitemia died significantly earlier than wild-type mice (around 95 days post infection vs 163 ± 12 days post infection). Thus, in the absence of Sepp1 gene expression, the host becomes more susceptible to T. congolense infection, in terms of parasite control ability and survival.

Selenoprotein P has two domains with respect to selenium content and functions. The N-terminal, selenium poor domain, encompassing two potential redox motifs, was predicted to have enzymatic (peroxidase) properties, while the C-terminal, selenium rich domain plays a role in selenium transport (21). To distinguish the function of the selenoprotein P domains during trypanosome infection, C57BL/6 mice with a deletion of the C-terminal domain of Sepp1 gene (Sepp1<sup>−/−</sup>/− mice) that have been recently generated (21) were used (Fig. 1). In contrast to Sepp1 KO mice, Sepp1<sup>−/−</sup>/− mice controlled T. congolense parasitemia as efficiently and survived as long as wild-type animals, suggesting that the enzymatic activity of selenoprotein P, and not its selenium transporter function, is important for African trypanosomiasis control.

3. Increased necrosis/apoptosis and oxidative stress occur in MCs from T. congolense-infected Sepp1 KO mice

Pathogenic insults in African trypanosome-infected mice result in necrosis/apoptosis in the liver, including Kupfer cells (17). In agreement, the shorter survival of T. congolense-infected Sepp1 KO C57BL/6 mice correlated with higher serum ALT values than in wild-type mice (Fig. 2A), indicating that hepatocytes from the former animals were more damaged. Moreover, the percentage of liver CD11b<sup>+</sup> MCs and of CD11b<sup>−</sup> cells undergoing apoptosis, as evidenced in flow cytometry by Annexin V staining, was higher in Sepp1 KO than in wild-type animals, both at the peak of parasitemia (day 7 post infection) and later on (day 90) during infection.
FIGURE 1. (A) Parasitemia and (B) survival at different times post *T. congolense* infection of wild-type, Sepp1<sup>α240-361</sup>, and Sepp1 KO C57BL/6 mice (*n* = 6). Beyond day 10 post infection, minor but similar waves of parasitemia were observed until the animals died in all experimental groups. *a* higher (*p < 0.05) than in wild-type- and Sepp1<sup>α240-361</sup>-infected mice.

(Fig. 2, B and C). The difference in apoptosis levels between infected wild-type and Sepp1 KO mice correlated with an increased accumulation of ROS within liver CD11b<sup>+</sup> MCs (Fig. 2, D and E). In contrast, *T. congolense*-infected Sepp1<sup>α240-361</sup> mice had similar levels of ALT (Fig. 2A), of apoptotic CD11b<sup>+</sup> and CD11b<sup>−</sup> cells (Fig. 2, B and C), and of ROS accumulation within CD11b<sup>+</sup> MCs (Fig. 2, F and G) as infected wild-type mice. Thus, selenoprotein P may play a role in reducing pathogenicity of *T. congolense* infection by protecting liver parenchymal and non-parenchymal cells from apoptosis/necrosis via its N-terminal domain-mediated enzymatic function. That the increased apoptosis/necrosis of liver MCs in Sepp1 KO mice affected the control of *T. congolense* growth was supported by the observation that treatment of wild-type mice at day 18 post infection with gadolinium chloride (32), which eliminates...
90% of liver MCs (Fig. 3) but only 20% of spleen MCs (not shown), abolished its ability to control the parasitemia and severely shortened its survival (22 ± 1 days vs 165 ± 25 days in nontreated mice; \( p < 0.01 \)) (Fig. 3).

4. Absence of Sepp1 does not affect cytokine environment and expression of other M2-associated, IL-10-dependent genes

Cytokine levels in the plasma and the expression of Ngfb, F13a1, and Ctss in MCs were measured during T. congolense infection in Sepp1 KO and Sepp1<sup>12240-361</sup> mice and compared with wild-type C57BL/6 mice. The IFN-\( \gamma \) and IL-10 titers were similar in all experimental groups both at the peak of parasitemia (day 7 post infection) and later on (day 90) during infection (Fig. 4). Moreover, no significant difference in the production of TNF-\( \alpha \) and NO, that are known to contribute to the control of trypanosome growth (8, 33), was observed between infected Sepp1 KO, Sepp1<sup>12240-361</sup>, and wild-type mice (not shown). The expression in the liver MCs of Ngfb, F13a1, and Ctss was similar in infected Sepp1 KO, Sepp1<sup>12240-361</sup>, and wild-type mice at days 9 and 90 post infection (not shown). Together, these data suggest that the increased susceptibility of T. congolense-infected Sepp1 KO mice is not reflected by a change in the immune environment or in the expression of the M2 genes potentially associated with lower severity of the disease tested.

Discussion

The gene expression profile of both human and mouse M2 is now relatively well studied. Yet, the mechanisms and downstream effector molecules by which these cells contribute to protective (34, 35) or detrimental (36, 37) type II immune response are poorly characterized. In contrast, it is clearly established that IL-10 is a major and multifunctional regulator of innate and adaptive immunity. By controlling the immune response, IL-10 creates an environment that favors the persistence of microbes and the development of chronic diseases. However, IL-10 can protect the host from the pathogenicity of diseases caused by exacerbated immune responses and, as such, avoids tissue or systemic lesions (38). Consequently, IL-10 inducible genes represent novel and more specific therapeutic options to intervene in the host's ability to mount a protective immune response during inflammatory/anti-inflammatory processes. In the present study, African trypanosome infection was used as a model to identify IL-10-dependent, M2-associated genes that could contribute to protection of the host against the pathogenicity associated with the disease.

Limited tissue damages and long survival in a host relatively resistant to African trypanosome infection result from a balance between (i) an inflammatory immune response that associates with the production of IFN-\( \gamma \), TNF-\( \alpha \), and NO as well as with the development of M1, and (ii) a counteracting anti-inflammatory response that includes IL-10, the development of M2, and the increased expression of M2-associated genes. In this study, we report the identification of IL-10-dependent genes that are associated with reduced severity of the disease and that contribute to the host's ability to resist the pathogenicity associated with the disease.

The expression in the liver MCs of Ngfb, F13a1, and Ctss was similar in infected Sepp1 KO, Sepp1<sup>12240-361</sup>, and wild-type mice at days 9 and 90 post infection (not shown). Together, these data suggest that the increased susceptibility of T. congolense-infected Sepp1 KO mice is not reflected by a change in the immune environment or in the expression of the M2 genes potentially associated with lower severity of the disease tested.

FIGURE 4. Plasma concentration of (A) IFN-\( \gamma \) and (B) IL-10 in wild-type, Sepp1<sup>12240-361</sup>, and Sepp1 KO C57BL/6 mice at days 7 and 90 post T. congolense infection (\( n = 5 \)), IFN-\( \gamma \) (\( * \)) or IL-10 (\( # \)) level higher (\( p < 0.05 \)) than in non infected mice (day 0 post infection).
expansion of Tregs. Both M2 (not shown) and Tregs (18) have been found to contribute to IL-10 production. Genetic polymorphism in host populations influences the progression both of natural (39–44) and experimental (45) African trypanosomiasis. Moreover, increased resistance is under the control of multiple genes that relate to the control of either the parasitemia or the pathogenic effects of the parasite. These genes remain largely unknown (46–48). However, quantitative trait loci studies performed in mice and cattle have revealed chromosomal regions linked to control of African trypanosomiasis. In particular in mice infected with *T. congolense*, 5 quantitative trait loci (17). In fact, Sepp1, Ngb, F13a1, and Cts1 can be considered as in vivo IL-10-dependent, M2-associated genes, the respective gene products of which could favor the control of the pathogenicity and hereby the resistance of *C57BL/6* mice to *T. congolense* infection. With the exception of the gene product of Cts1, which contributes to Ag presentation and matrix degradation (52, 53), the other genes encode proteins controlling inflammatory/anti-inflammatory processes (54–58).

Despite producing higher levels of IL-10 than *C57BL/6* mice, BALB/C mice, that are highly susceptible to *T. congolense* infection and die within 10 ± 3 days post infection, did not up-regulate the expression of Sepp1, Ngb, F13a1, and Cts1 in MCs (not shown). It has been suggested that infected BALB/C mice are not responsive to the effect of IL-10 (17). In fact, *T. congolense*-susceptible BALB/c mice, that do not control the first peak of parasitemia, produce simultaneously IFN-γ and IL-10 in the early stage of infection while resistant *C57BL/6* mice change from a predominant IFN-γ environment to a predominant IL-10 environment after the control of the first peak of parasitemia (59). Thus, we cannot exclude that the mixed IFN-γ and IL-10 response in BALB/C mice in the early stage of infection impairs the efficient activation of MCs, including up-regulation of Sepp1, Ngb, F13a1, and Cts1 expression.

Selenoprotein P is the major transporter of selenium, a trace element required for normal development (60, 61). This secreted glycoprotein is almost exclusively synthesized by the liver, but local production in virtually all tissues has been described (62–64), including M2 elicited in several infection and cancer models (2). Selenoprotein P plasma level is depressed in a number of human pathologies including cirrhosis and Crohn’s disease (65, 66). Moreover, human and/or murine Sepp1 gene promoter activity is impaired by IFN-γ, TNF-α, and IL-1β (67) while induced by TGF-β (68) and IL-10 (2) suggesting a differential regulation of selenoprotein P expression during type 1 or 2 inflammatory reactions. In addition, selenoprotein P was proposed to function in anti-oxidant defense, as a scavenger of peroxynitrite or as a phospholipid hydroperoxide thiol peroxidase (26, 28, 69). Accordingly, selenoprotein P expressed in human pancreatic cancer cells associates with resistance to chemotherapy by suppressing the induction of ROS (70). Hereby, its pH-dependent heparin-binding property suggests that selenoprotein P binds to host cell membranes in areas of inflammation that typically have low pH, preventing cell injury induced by ROS (70–72).

Currently, a documented role for selenoprotein P in M2 and in infectious diseases is lacking. In an attempt to validate our approach to identify IL-10-dependent, M2-associated genes contributing to the control of the severity of African trypanosomiasis, Sepp1 KO mice, and Sepp1Δ240-361 mice-expressing selenoprotein P truncated of the selenium transporter domain but retaining the domain exerting anti-oxidant activity-in the resistant *C57BL/6* background were infected with *T. congolense*. It has been shown that in Sepp1 KO or Sepp1Δ240-361 mice, severe neurological dysfunction occurs in animals fed a selenium-deficient diet. Yet, feeding 0.25 mg selenium/kg largely prevents this phenotype in Sepp1 KO or Sepp1Δ240-361 mice (20–22, 73). In this context, it is important that in our experiments, Sepp1 KO and Sepp1Δ240-361 mice were supplied with 10 times the normal nutritional requirement, 1 mg selenium/kg, preventing pathological phenotype and selenium depletion in most tissues including the liver. Our data show that in *T. congolense*-infected wild-type animals, the expression of Sepp1 mRNA gradually increased in liver CD11b+ MCs after the clearance of the first peak of parasitemia. Concomitantly, it was not affected in CD11b− cells. Of interest, the mRNA expression level of other selenoproteins tested (thioredoxin reductases 1–3 and glutathione peroxidases 1–4) in CD11b+ cells and CD11b+ MCs from the liver was not up-regulated in *T. congolense* infected wild-type and Sepp1 KO mice (not shown). These data suggest that selenoprotein P is the major selenoprotein induced in infected mice and that none of the other selenoproteins tested compensates, in MCs, for the loss of selenoprotein P in Sepp1 KO mice. *T. congolense* infection is more pathogenic in Sepp1 KO mice. The increased parasitemia and reduced survival of these animals as compared with wild-type mice correlate with increased liver injury, including apoptosis/necrosis of hepatocytes and non-parenchymal cells. Of note, the increased susceptibility of Sepp1 KO mice to *T. congolense* infection does not associate with a change in cytokine environment or in the expression of IL-10-dependent M2 genes associated with resistance to the disease (Ngb, F13a1, and Cts1). In addition, while all IL-10 KO mice died after the first peak of parasitemia, only half of the Sepp1 KO mice died at that moment, suggesting that the effect of IL-10 on resistance to *T. congolense* is not restricted to the sole induction of IL-10-dependent genes identified in the present study.

In contrast to Sepp1 KO mice, Sepp1Δ240-361 mice controlled *T. congolense* infection as efficiently as wild-type animals, suggesting that the anti-oxidant activity of selenoprotein P is important to avoid tissue damage and ensure survival. In agreement, in *T. congolense*-infected mice, liver MCs from Sepp1 KO mice, but not from Sepp1Δ240-361 mice, accumulate more ROS than wild-type mice. Although ROS can limit trypanosome growth (29, 30), parasitemia increases in Sepp1 KO-infected mice during the first, most aggressive wave of parasitemia. Since liver MCs were more prone to undergo apoptosis/necrosis in the latter animals, and since gadolinium chloride treatment, which eliminates 90% of liver MCs prone to undergo apoptosis/necrosis cannot be excluded.

In summary, by analyzing the gene profile in M2-oriented MCs in *T. congolense*-infected mice, we have identified IL-10-dependent genes that could prevent the pathogenicity and, thus, contribute to the resistance of the host to the disease. These genes code for proteins potentially involved in the control of inflammatory processes. One of these genes coding for selenoprotein P was found...
essential to control the pathogenic effect of the parasite including excessive production of ROS as well as destruction of liver MCs and hepatocytes. In a more general context, M2 genes that contribute to the limitation of severity of African trypanosomiasis infection may represent targets for therapeutic intervention aiming to limit potentially harmful inflammatory responses.

Acknowledgments

We thank Drs. Patrizia Loi and Véronique Flamand (Institut de Biologie et Médecine Moléculaires, Université Libre de Bruxelles, Belgium) for measuring AL/Tasparte aminotransferase levels.

Disclosures

The authors have no financial conflict of interest.

References


55. Wei, R., and G. M. Jonakait. 1999. Neurotrophins and the anti-inflammatory agents interleukin-4 (IL-4), IL-10, IL-11 and transforming growth factor-


