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Inhibition of In Vitro Erythropoiesis by Soluble Mediators in *Plasmodium chabaudi* AS Malaria: Lack of a Major Role for Interleukin 1, Tumor Necrosis Factor Alpha, and Gamma Interferon

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By using erythropoietin-dependent proliferation of splenic erythroid cells as an in vitro erythropoiesis model system, we demonstrate that spleen cells from *Plasmodium chabaudi* AS-infected C57BL/6 mice potently inhibited erythroid cell proliferation. Inhibitory activity was detected in spleen cell conditioned media (SPCM) prepared from infected mice but not from uninfected mice. The inhibitory activity in SPCM was characterized as being heat sensitive, macromolecular, and host derived. The inhibitory activity was not reversed by increasing the erythropoietin concentration and was found to be specific for the late erythroid lineage. Mouse strains, which differ in their resistance to *P. chabaudi* AS infection, produced and responded to the inhibitory activity to a similar extent. Putative immune mediators, interleukin 1α, interleukin 1β, and gamma interferon, were found to be potent inhibitors of erythroid cell proliferation. However, antibody neutralization experiments failed to demonstrate a major role for these cytokines in the inhibitory activity of SPCM. Our results suggest that the elaboration of inhibitor(s) of erythropoiesis in hemopoietic organs of *Plasmodium*-infected mice may impair erythroid regeneration. The identity of the inhibitory mediator(s) is presently unknown but is distinct from interleukin 1, tumor necrosis factor alpha, and gamma interferon.

Anemia together with cerebral malaria accounts for the highest proportion of deaths due to severe malaria in Africa (5). The pathophysiologic mechanisms of the anemia of malaria are complex and multifactorial (13, 15). Since *Plasmodium* parasites reside within host erythrocytes, schizogony and the subsequest release of merozoites inevitably lead to intravascular hemolysis. In addition, it is thought that erythrophagocytosis of noninfected erythrocytes (RBC) and a depressed erythropoietic response contribute to the development of anemia (8, 14, 15). Morphologic studies of bone marrow smears obtained from patients as well as mice with malaria have provided cytologic evidence for dyserythropoiesis (2, 4, 14). A detailed analysis of erythroblast cell kinetics using quantitative ^{14}C autoradiography in bone marrow samples of patients with acute *Plasmodium falciparum* malaria revealed a reduction in proliferative rates in all erythroblast stages and a significant intramedullary deletion in the polychromatophilic erythroblast compartment (4).

The mechanisms which account for these changes are not well understood. It has been postulated that host-derived factors, such as tumor necrosis factor (TNF), produced in response to parasites may cause the depression in erythropoiesis (2). Recently, Miller and colleagues (11) reported that a soluble factor released from cultured bone marrow and spleen cells of mice infected with *Plasmodium berghei* or *Plasmodium vinckei* was able to depress the in vitro erythropoietin (Epo)-induced proliferation of enriched splenic erythroid precursors. The identity of this soluble mediator and its possible contribution to the development of anemia in vivo are unknown.

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In a preliminary report, we demonstrated that a soluble inhibitory activity is also produced by bone marrow and spleen cells derived from *Plasmodium chabaudi* AS-infected mice (22). In the present investigation, we have characterized the biological and physicochemical properties of the inhibitory activity present in conditioned media of spleen cells from infected C57BL/6 mice. Furthermore, we demonstrate that inhibition of Epo-induced proliferation of splenic precursors by conditioned media from *P. chabaudi* AS-infected mice is not mediated by either interleukin-1 (IL-1), TNF alpha (TNF-α), or gamma interferon (IFN-γ), cytokines which have been suggested previously to play major roles in the suppression of erythropoiesis during malaria (2, 11).

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MATERIALS AND METHODS

Animals and experimental infection. C57BL/6, BALB/C, AKR, and DBA/2 mice were purchased from Charles River (St. Constant, Quebec, Canada), and A/J and C3H/HeJ mice were from the Jackson Laboratory (Bar Harbor, Maine). Six- to 8-week-old, age- and sex-matched mice were routinely used for infection and for phenylhydrazine injection. Experimental mice were infected intraperitoneally with 10^6 *P. chabaudi* AS parasitized RBC (PRBC) as described previously (23).

Preparation of SPCM. Spleens were obtained aseptically from normal and infected mice at peak parasitemia (typically, day 7 postinfection). Cell suspensions were prepared by passing minced spleens through a sterile wire mesh and washing them three times with complete medium consisting of Iscove's modified Dulbecco's medium (IMDM; GIBCO Laboratories, Grand Island, N.Y.) supplemented with 5% fetal calf serum

(Hyclone Laboratories, Logan, Utah) 0.12% gentamycin (Schering Canada, Montreal, Quebec), 2 mM glutamine, and 5×10^{-5} M 2-mercaptoethanol. Total viable cell counts were determined with a hemacytometer after appropriate dilution with trypan blue. Spleen cell conditioned media (SPCM) were prepared by culturing the spleen cells at a density of 5×10^6 cells per ml for 2 days in a well-humidified CO_2 incubator. Supernatants were harvested and stored at −20°C until used.

Inhibition assay for Epo-induced proliferation. The inhibition assay for Epo-induced proliferation was performed essentially as described by Krystal (7). Spleen cells were obtained from mice made anemic by two consecutive daily phenylhydrazine injections (60 mg/kg of body weight per day). Unless otherwise indicated, spleen cells were prepared from phenylhydrazine-treated C57BL/6 mice. Aliquots of 100 ml of spleen cells at 5×10^6 cells per ml in complete IMDM medium with 20% fetal calf serum and 100 mU of recombinant human Epo (Genetics Institute, Cambridge, Mass.) per ml were distributed into 96-well plates. SPCM samples were added at 20% (vol/vol), and each sample was assayed in triplicate. Cultures were incubated for 24 h. Two hours before harvesting, 20 μl of IMDM containing 1 μCi of [^{3}H]thymidine (ICN, Montreal, Quebec, Canada) was added to each well. DNA synthesis was measured by liquid scintillation counting of cells harvested on glass fiber discs with an automatic cell harvester (Skatron, Inc., Sterling, Va.). Inhibition of cell proliferation was assessed by dividing the mean counts of cultures with SPCM by the mean counts of cultures with medium alone. In some cases, lipopolysaccharide (LPS; *Escherichia coli* O55:B5; Difco Laboratories, Detroit, Mich.), polymyxin B (25 mg/ml), washed normal RBC (NRBC) or PRBC, or soluble extracts of NRBC or PRBC were added directly to the splenic erythroid cultures. Soluble extracts were prepared by incubating NRBC or PRBC at 10^8 cells per ml in Hanks balanced salt solution at 37°C in roller tubes for 24 h (1). Supernatants were cleared by boiling for 5 min, centrifuged again, and passed through a 0.2-μm-pore-size filter (Gelman, Ann Arbor, Mich.) and stored at 4°C.

Cytokines and antibodies. Recombinant human IL-1α, IL-1β, and murine IL-4 were kindly provided by S. Gillis (Immunex Corp., Seattle, Wash.). Recombinant human IL-6 (2×10^8 U/mg), recombinant murine TNF-α (4×10^7 U/mg), and recombinant murine IFN-γ (4.5×10^6 U/mg) were purchased from Boehringer Mannheim (Montreal, Quebec, Canada; IL-6) and Genzyme (Cambridge, Mass.; TNF-α and IFN-γ). Cytokine preparations were diluted in IMDM and added to cultures as detailed above. DB-1, a murine anti-rat IFN-γ monoclonal antibody was generously given by P. van der Meide (TNO Primate Centre, Rijswijk, The Netherlands). Polyclonal sheep anti-mouse IL-1α and goat anti-mouse IL-1β antisera were kindly provided by H. Ziltener (The Biomedical Research Centre, Vancouver, British Columbia, Canada). Antibodies were used at concentrations which completely inhibited 10 U of IFN-γ per ml and 10 pg of IL-1 per ml. Higher concentrations of antibodies gave similar results.

Hemopoietic colony assay. Bone marrow cells obtained from femora of C57BL/6 mice were plated at a density of 5×10^5 cells per ml in a semisolid medium as described previously (12). This medium consists of 0.8% methylcellulose, 30% fetal calf serum, 10% WEHI-3 CM (as a source of IL-3), 2 U of Epo per ml, 0.1 mM hemin (Eastman Kodak Co., Rochester, N.Y.), 2 mM glutamine, and 5×10^{-5} M 2-mercaptoethanol in IMDM. Test supernatants or medium was added at 20% (vol/vol). Colonies were scored in situ after 6 days. The CFU-erythroid (CFU-E) assay was done as described previously (22, 23).

Statistics. Differences between groups of cultures assayed at the same time were analyzed by Student's *t* test. A probability of less than 0.05 is reported as significant. The data shown are from representative experiments which have been repeated at least twice.

RESULTS

To evaluate the inhibitory activity of spleen cells from *P. chabaudi* AS-infected mice on erythropoiesis in vitro, we utilized a previously described proliferation assay using splenic erythroid cells derived from mice rendered anemic by phenylhydrazine injection as responder cells (PHZ-SP) and recombinant human Epo as mitogen (7, 11). This cell population has been shown recently to contain greater than 90% erythroid precursor cells by its morphology and high-level expression of transferrin and Epo receptors (21).

The effect of spleen cells from uninfected and infected C57BL/6 mice at peak parasitemia on the proliferation of splenic erythroid precursor cells was determined by using this in vitro assay. Figure 1a illustrates that spleen cells from *P. chabaudi* AS-infected mice added at different concentrations (to give a range of spleen cell/responder cell ratios of 1:10 to 2:1) almost completely abrogated the proliferative responses of PHZ-SP to Epo ($P < 0.0001$). In contrast, normal spleen cells were not inhibitory at a 1:5 ratio but, at higher concentrations (1:1 to 2:1), modestly but significantly inhibited proliferative responses ($P < 0.001$). However, at cell ratios greater than 1:50, spleen cells from infected mice significantly inhibited PHZ-SP proliferation in comparison with spleen cells from normal mice ($P \leq 0.001$). We next determined whether soluble mediators released by spleen cells from *P. chabaudi* AS-infected mice are responsible for the inhibition. SPCM prepared from *P. chabaudi* AS-infected spleen cells were found to significantly inhibit the proliferative response of PHZ-SP ($P < 0.001$), while SPCM from normal, uninfected spleen cells failed to significantly inhibit the response relative to the medium control (Fig. 1b). It is notable that the addition of SPCM from infected mice inhibited proliferative responses by approximately 10-fold less than the addition of spleen cells from infected mice.

Characterization of the physicochemical properties of the inhibitory activity in SPCM from infected C57BL/6 mice showed that, after treatment at 56°C for 30 min, there was no significant loss of inhibition. Inhibitory activity was completely lost after boiling for 1 h. Ultrafiltration of SPCM by using a membrane with a 10-kDa-molecular-mass cutoff resulted in the enrichment of inhibitory activity in the concentrate of SPCM prepared from infected but not from control mice (data not shown). Passage of the concentrate on a Sephadex G-25 column and subsequent assay of the fractions showed that most of the inhibitory activity was in the void volume (data not shown). These results therefore demonstrate that the inhibitory activity is heat sensitive and macromolecular. The addition of polymyxin B (25 μg/ml) had no effect on the inhibitory activity (25 and 32% of control response with and without polymyxin B, respectively). This observation together with the heat sensitivity of the activity demonstrates that inhibition is not caused by LPS contamination. The direct addition of PRBC or soluble parasite extracts did not suppress erythroid cell proliferation compared with NRBC or extracts. Therefore, the inhibitory activity is host and not parasite derived.

To estimate the relative concentration of the inhibitory activity, SPCM from infected C57BL/6 mice were serially diluted in complete medium and assayed for inhibitory activity. There was a dose-dependent inhibition, with concentrations of only 20 and 10% (vol/vol) resulting in significant inhibition

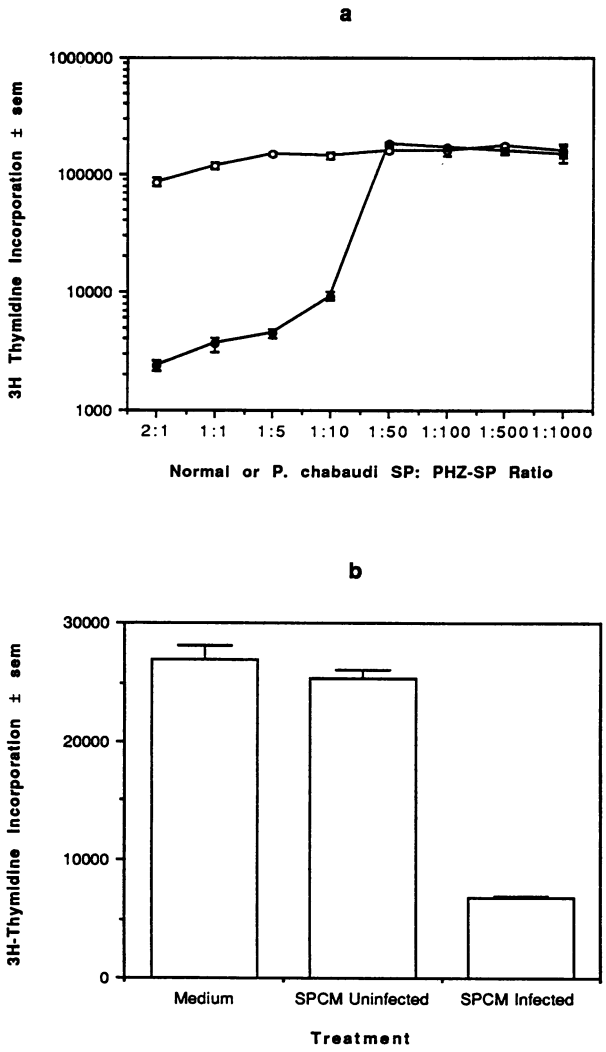

FIG. 1. (a) Inhibition of Epo-induced proliferation of splenic erythroid cells (5×10^5 per well) by spleen cells from normal (○) and *P. chabaudi* AS-infected (●) mice. Thymidine incorporation values of PHZ-SP with or without Epo (100 mU/ml) were 169,227 ± 6,876 and 1,672 ± 231 cpm, respectively. (b) Inhibition of Epo-induced proliferation of splenic erythroid cells by SPCM (used at 20% [vol/vol]) from *P. chabaudi* AS-infected mice sem, standard error of the mean. SP, spleen cells.

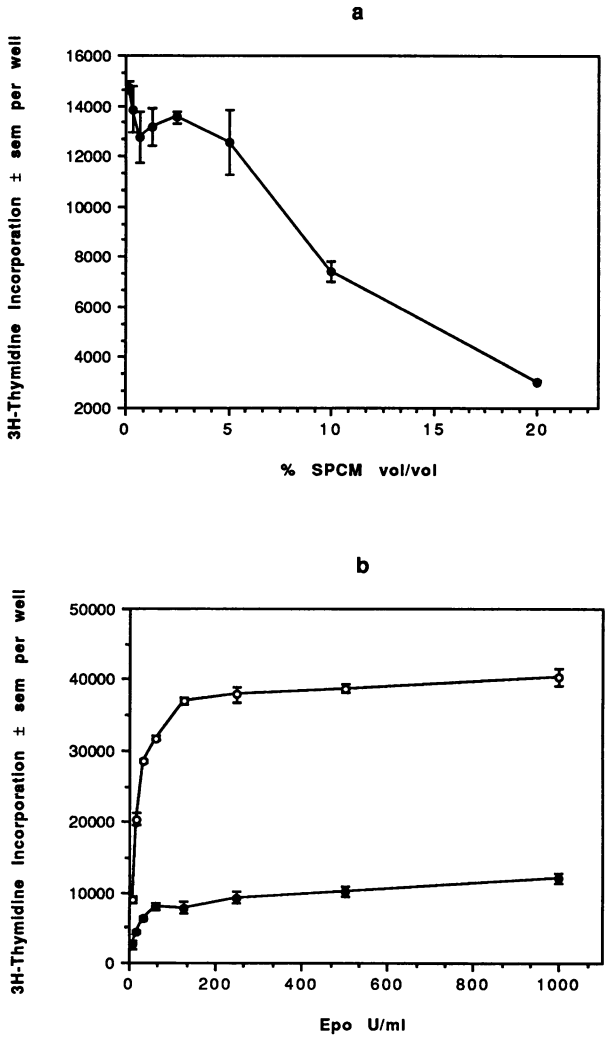

FIG. 2. Dose response studies of SPCM inhibitory activity. (a) Dose response of SPCM inhibition at 10 mU of Epo per ml; (b) effect of increasing Epo concentrations on inhibition with SPCM (20% [vol/vol]). sem, standard error of the mean.

(Fig. 2a; $P = 0.0004$ and 0.0007, respectively). The effect of SPCM added at a constant concentration (20% [vol/vol]) with increasing concentrations of Epo on the proliferative response of splenic erythroid cells was also investigated. Figure 2b illustrates that proliferative responses of erythroid progenitors were significantly inhibited even at high Epo concentrations. Proliferative responses in cultures containing SPCM were 20 to 30% of control responses at all Epo concentrations tested. These results suggest that inhibitory activity in SPCM from infected C57BL/6 mice is present in low concentrations and that high concentrations of Epo fail to reverse the inhibition.

Our previous results demonstrated that the inhibitory activity in SPCM was erythroid specific, that is, it inhibited CFU-E formation and erythroblast proliferation but not CFU-culture (CFU-C) and monocyte-macrophage proliferation (22). We extended this observation by examining the effects of SPCM on IL-3 and Epo-driven colony formation of bone marrow cells of the myeloerythroid lineage. Table 1 shows that, in comparison with control cultures incubated with medium alone or cultures incubated with SPCM from uninfected mice, SPCM from infected mice did not inhibit burst-forming unit-erythroid (BFU-E), CFU-granulocyte-macrophage (CFU-GM), or multipotential CFU-granulocyte-erythroid-macrophage-megakaryocyte (CFU-GEMM) colony formation by bone marrow precursor cells. However, the addition of SPCM from infected mice significantly inhibited CFU-E colony formation ($P < 0.05$). These observations suggest that the inhibitory activity is effective primarily in the late erythroid compartment.

Our previous studies have shown an impairment of splenic erythropoiesis in genetically susceptible A/J mice (23). We reasoned that an increased sensitivity to and/or production of the inhibitory activity may explain this observation. We tested a standard SPCM preparation from infected C57BL/6 mice on splenic erythroid cells from different inbred mouse strains. Inbred mouse strains which have been previously typed as resistant (DBA/2 and C57BL/6) or susceptible (BALB/c and

TABLE 1. Effect of SPCM from *P. chabaudi* AS-infected C57BL/6 mice on IL-3 and Epo-driven in vitro colony formation by bone marrow and spleen cells

Treatment[a]	Mean no. of colonies ± SEM[b]			
	BFU-E	CFU-GM	CFU-GEMM	CFU-E
Medium alone	31 ± 3	292 ± 6	7 ± 1	805 ± 33
SPCM from uninfected mouse	29 ± 1	302 ± 7	8 ± 1	795 ± 39
SPCM from infected mouse				
No. 1	30 ± 1	297 ± 5	12 ± 1	403 ± 72[c]
No. 2	35 ± 3	309 ± 8	12 ± 2	407 ± 57[c]
No. 3	33 ± 2	278 ± 18	12 ± 1	487 ± 95[c]

[a] SPCM was prepared from one uninfected and three individual *P. chabaudi* AS-infected C57BL/6 mice as described in Materials and Methods. Infected animals are designated numerically. SPCM from infected mice exhibited significant inhibitory activity on splenic erythroid precursor cells incubated in the presence of 100 mU of Epo per ml.

[b] Colony counts per 2×10^5 bone marrow or spleen cells from C57BL/6 mice plated at 1 ml per tissue culture dish in the presence of 20% (vol/vol) SPCM from uninfected or infected C57BL/6 mice. Results are presented as mean number of colonies ± standard error of the mean for triplicate cultures.

[c] $P < 0.05$.

A/J) to *P. chabaudi* AS were used (18). The responsiveness to the inhibitory activity did not differ among the strains studied (49, 60, 61, and 60% of control levels, respectively). We also prepared SPCM from these four inbred strains at peak parasitemia after infection with 10^6 *P. chabaudi* AS PRBC and tested for inhibitory activity on splenic erythroid precursor cells from C57BL/6 mice. There was no striking difference in production of inhibitory activity among the strains examined (26, 31, 17, and 23% of control levels, respectively).

To identify candidate cytokines which may mediate the inhibitory activity in SPCM, several recombinant cytokines were added directly to the cultures. As shown in Fig. 3, IFN-γ, IL-1α, IL-1β, and IL-4 inhibited proliferative responses maximally at concentrations of 10 U/ml, 10 pg/ml, 10^3 pg/ml, and 10^4 pg/ml, respectively. TNF-α, IL-6, and transforming growth factor beta (not shown) were not found to be inhibitory over a wide range of concentrations tested. Next, antibody neutralization experiments were carried out to ascertain the contribution of IL-1 and IFN-γ to the inhibitory activity of SPCM from infected mice (Table 2). DB-1, a mouse anti-rat IFN-γ, slightly increased the proliferative response in three of three SPCM preparations ($P < 0.05$). These SPCM preparations were found to contain low but detectable levels of IFN-γ (2 to 5 U/ml). Nonetheless, these cultures were significantly inhibited compared with cultures containing control SPCM and DB-1 ($P < 0.001$). Polyclonal antisera to mouse IL-1α and IL-1β had no significant effect on inhibitory activity. The addition of anti-IL-1 and DB-1 together did not increase thymidine labelling above that in DB-1-containing cultures. It therefore appears that neither IL-1 nor IFN-γ, alone or in combination, could account for a majority of the inhibitory activity of SPCM.

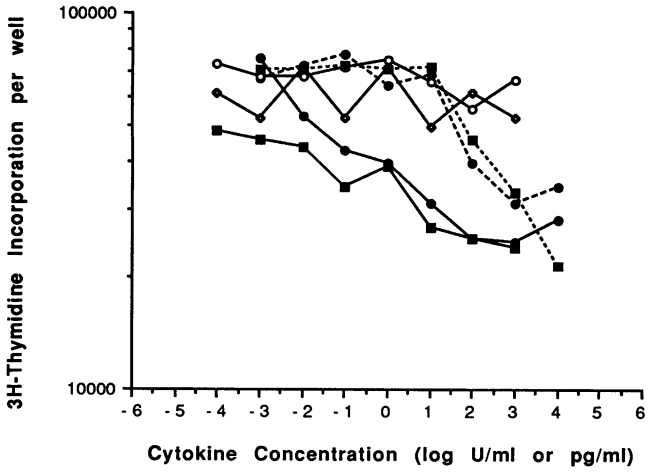

FIG. 3. Inhibitory activity of recombinant cytokines on Epo-induced proliferation of PHZ-SP. The dose range used for IL-1α (-●-), IL-1β (--●--), and IL-4 (--■--) was 10^{-2} to 10^4 pg/ml, and that for IFN-γ (-■-), TNF-α (-○-), and IL-6 (-◇-) was 10^{-3} to 10^3 U/ml. Epo was added at 100 mU/ml.

DISCUSSION

In the present report, we demonstrate that spleen cells recovered from mice infected with *P. chabaudi* AS at peak parasitemia completely suppressed the Epo-induced proliferative response of enriched erythroid precursors. This suppression appears to be due to the presence of an inhibitory activity in SPCM prepared from *P. chabaudi* AS-infected mice. Our results confirm and extend earlier work by Miller and colleagues (11) which demonstrated the presence of soluble inhibitor(s) of erythropoiesis in conditioned media from spleen cells of mice infected with the more-virulent *P. berghei* and *P. vinckei*. Their results and ours suggest that, at least in mice, the production of this soluble inhibitory activity may be a universal feature of malaria infection. However, the relative potency of spleen cells over SPCM in inhibiting proliferative responses argues for additional mechanisms which may require cell contact or operate within a short range.

We performed a series of experiments to characterize the inhibitory activity in SPCM from *P. chabaudi* AS-infected mice. The possibility that LPS contamination contributed to the inhibition was excluded by demonstrating the heat sensitivity of SPCM and the lack of effect of polymyxin B. Another concern which has been addressed in our study is the possibility of nonspecific cytotoxicity causing the decreased thymidine incorporation in SPCM-containing cultures; this concern has also been excluded. Examination of the inhibitory activity of SPCM on bone marrow precursor cells of various lineages indicates that it is specific to cells of late erythroid lineage. As we have previously shown, SPCM inhibited proliferation of spleen cells from phenylhydrazine-treated mice and CFU-E colony formation but did not inhibit macrophage colony-stimulating factor-driven bone marrow cell proliferation and CFU-C colony formation (22). As demonstrated here, SPCM also failed to inhibit the early erythroid progenitor, BFU-E, and the multipotential progenitor, CFU-GEMM (Table 1). This apparent lineage specificity of inhibition by SPCM from *P. chabaudi* AS-infected mice thus excludes the possibility of a nonspecific cytotoxic or cytostatic effect. Furthermore, direct observation of splenic erythroid cells incubated with SPCM over a 24-h period failed to demonstrate the significant loss of viability determined by trypan blue exclusion (data not shown).

The identity of the factor(s) which mediates the inhibitory activity of SPCM is not yet known. It has been postulated that a Mac 1^+, adherent cell, presumably a monocyte-macrophage, secretes cytokines, such as IL-1 and TNF, which inhibit erythropoiesis (11). Both IL-1α and IL-1β have been shown to inhibit Epo-induced proliferation of anemic mouse spleen cells

TABLE 2. Effects of neutralizing antibodies to IFN-γ and IL-1 on the inhibitory activity of SPCM

Treatment[a]	Activity (cpm [mean ± SEM]) after antibody treatment with:					
	Medium	Anti-IL-1α	Anti-IL-1β	Anti-IL-1α + anti-IFN-γ	Anti-IL-1β + anti-IFN-γ	Anti-IFN-γ
Medium control	48,816 ± 3,368	48,371 ± 828	48,216 ± 555	47,510 ± 1,096	47,191 ± 1,199	46,159 ± 1,328
SPCM from uninfected mouse	38,809 ± 2,147	38,880 ± 439	43,463 ± 1,934	44,334 ± 1,108[b]	37,186 ± 361[b]	39,115 ± 1,469[c]
SPCM from infected mouse						
No. 1	13,635 ± 1,910[d]	11,675 ± 182	12,960 ± 402	21,244 ± 2,336[b,e]	17,999 ± 1,079[b,e]	19,673 ± 308[c,d,e]
No. 2	16,950 ± 519[d]	16,307 ± 1,824	18,891 ± 800	21,284 ± 1,484[b,e]	18,856 ± 396[b,e]	20,135 ± 1,230[c,d,e]
No. 3	16,588 ± 673[d]	19,163 ± 2,167	17,661 ± 1,381	18,341 ± 1,314[b,e]	17,449 ± 450[b,e]	19,232 ± 457[c,d,e]

[a] IFN-γ titers by ELISA (19): uninfected, <1 U/ml; infected mouse no. 1, 5 U/ml; infected mouse no. 2, 2.2 U/ml; infected mouse no. 3, <1 U/ml.
[b] $P < 0.001$ compared with uninfected SPCM within same column.
[c] $P < 0.001$ compared with control, uninfected cultures treated with anti-IFN-γ.
[d] $P < 0.05$ compared with medium control.
[e] $P > 0.1$ compared with anti-IFN-γ treated cultures within same row.

(17) (Fig. 3). Alternatively, IFN-γ production by activated T cells and/or NK cells may be responsible for the inhibitory activity. Indeed, IFN-γ has been implicated as a major mediator of erythroid suppression in patients with aplastic anemia and rheumatoid arthritis (9, 24). We have previously demonstrated that spleen cells from *P. chabaudi* AS-infected C57BL/6 mice secrete high levels of IFN-γ in response to antigenic or concanavalin A stimulation (19). However, neutralizing antibodies to IL-1 and IFN-γ are ineffective in reversing inhibition by SPCM. Furthermore, antibody and complement depletion of Thy-1^+ cells from spleen cell suspensions, which almost totally eliminated IFN-γ in SPCM, does not significantly affect inhibitory activity (unpublished observations). These results indicate that IL-1 and IFN-γ do not play an essential role in the inhibitory activity of SPCM. However, we cannot discount the possibility that the small quantities of these cytokines may cooperate with other as-yet-unidentified cytokines to mediate the inhibitory activity. It is therefore necessary to consider other cytokines as candidate inhibitory factors. TNF-α, which inhibits erythropoiesis in vivo (6, 20), has no inhibitory activity in the in vitro assay used in this study (17) (Fig. 3). Combined treatment with IFN-γ and TNF-α did not significantly reduce proliferative responses compared with IFN-γ alone. Furthermore, neutralizing antibodies to TNF-α added together with anti-IFN-γ (at concentrations which neutralized 10 U of each cytokine per ml added together) had no significant effect on SPCM activity (unpublished results). Recently, it has been demonstrated that TNF-α exerts a negative effect on human CFU-E growth indirectly by inducing IFN-β production by stromal fibroblastic cells (10). In addition, IFN-β has been implicated in natural suppressor activity and is spontaneously produced by spleen and bone marrow cells from mice undergoing graft-versus-host disease, postirradiation, and neonatal immunosuppressive states (3). Indeed, in our hands, murine IFN-α/β was found to be inhibitory at a concentration range of 100 to 1,000 U/ml. However, a polyclonal antiserum against IFN-α/β (at concentrations which neutralized 500 U/ml) was unable to reverse the effects of SPCM (unpublished observation).

How relevant is the inhibitory activity in vivo? Histological examination of spleen tissue from malaria-infected mice revealed macrophages engorged with malarial pigment closely apposed to developing erythroblasts in the red pulp (our unpublished observations). It is, therefore, likely that sufficiently high concentrations of the inhibitor can be achieved in the immediate microenvironment of the developing erythroblasts. Previous studies have shown that spleen cells produce the inhibitor transiently at the time of peak parasitemia (22). Inhibitory cytokines produced during this critical period may delay or decrease the reticulocytic response which follows within 2 to 4 days. Comparison of the production and responsiveness to the inhibitory activity among resistant and susceptible inbred mouse strains, however, failed to demonstrate any significant strain differences. This observation implies that neither the production of nor the responsiveness to the inhibitory activity can be directly correlated with the survival or death of the infected mice. Our previous in vivo studies demonstrated a concurrent decrease in erythropoietic activity in the bone marrow and increase in the spleen at the time of peak parasitemia (23). However, maximal inhibitory activity occurred in both bone marrow and SPCM at the time of peak parasitemia (22). Thus, this apparent paradox implies that additional mechanisms (e.g., preferential migration and homing to the spleen and differences in hemopoietic stromal support) regulate the erythropoietic activity in the two organs. Identification of the cytokine and the cellular requirements for inhibition of erythropoiesis represents an initial step to further our understanding of the complex pathophysiology of anemia which occurs during malaria and other infections and inflammatory states.

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