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Antimicrobial Properties of Kupffer Cells

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To characterize the antimicrobial activities of Kupffer cells, I harvested macrophages from livers with a technique involving perfusion with collagenase and DNase. Ninety-nine percent of glass-adherent cells had typical macrophage morphology, 99% were esterase positive, and 60% phagocytosed opsonized zymosan when challenged with four particles per macrophage. *Toxoplasma gondii* multiplied within Kupffer cells from unmanipulated mice, but multiplication was intermediate between that observed in highly permissive peritoneal macrophages and highly activated macrophages. Intravenous injection of heat-killed *Propionibacterium acnes*, a stimulus known to activate macrophages in other compartments, resulted in a uniform, highly activated population of liver macrophages. Kupffer cells from *P. acnes*-injected mice were capable of generating reactive oxygen intermediates as shown by reduction of Nitro Blue Tetrazolium during phagocytosis of *T. gondii* or opsonized zymosan. In contrast, intravenous *P. acnes* injection did not activate spleen macrophages. Intravenous injection of *P. acnes* into athymic mice activated Kupffer cells, which suggested that T cells were not essential for this response. Kupffer cells were not activated in mice with latent *Toxoplasma* infection or during acute *Giardia muris* infection. Ordinarily, Kupffer cells became highly permissive for *T. gondii* during 48 h in culture, but inclusion of recombinant murine gamma interferon maintained their moderate inhibitory activity.

Antimicrobial properties of Kupffer cells are not well understood. For example, when it was observed that susceptibilities of different strains of mice infected with *Leishmania donovani* were associated with growth of *L. donovani* within liver macrophages in vitro (5), the conclusion was that liver macrophages were important for host defense. In contrast, others (14) found that Kupffer cells had little innate antimicrobial activity, expressed against *L. donovani* or *Toxoplasma gondii*. In some studies, rat (2, 3, 16) or mouse (9) Kupffer cells stimulated in vitro exhibited oxidative respiratory bursts and the magnitudes of the bursts were greater if the animals had received systemically administered inflammatory stimuli before Kupffer cells were harvested (2, 9, 10, 16). In other studies (14), Kupffer cells were incapable of oxidative respiratory bursts.

In this work, I further characterized antimicrobial activities of Kupffer cells. I obtained Kupffer cells with a method that left cellular antigens intact and allowed me to study Kupffer cells immediately after they adhered to glass (22), which offered advantages over earlier methods. I studied the interactions of Kupffer cells with *T. gondii*, their activity after injection of heat-killed *Propionibacterium acnes*, and their response to recombinant gamma interferon in vitro. To explore the possibility that an infection localized to the gastrointestinal tract might affect Kupffer cells, I studied Kupffer cell antimicrobial activity during intestinal infection with *Giardia muris*.

(Parts of the work were presented at the 25th Interscience Conference for Antimicrobial Agents and Chemotherapy, Minneapolis, Minnesota, 30 September to 2 October, 1985.)

MATERIALS AND METHODS

Mice. Specific-pathogen-free BALB/c AnN Cr, athymic NCr-*nu*/*nu*, and athymic NCr-*nu*/+ female mice (National Cancer Institute, Frederick, Md.) were used at 6 to 8 weeks of age (average weight, 20 g). All mice were maintained in specific-pathogen-free facilities in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Sera from sentinel mice were free of antibody to murine hepatitis virus, Sendai virus, and *Mycoplasma pulmonis*.

Kupffer cells. Kupffer cells were harvested as previously described, with minor modifications (20, 22, 23). Briefly, mice were sacrificed, and the livers were perfused through the superior mesenteric vein with Hanks balanced salt solution containing 0.05% collagenase (139 U/mg; LSO 4182, Organon Teknika, Malvern, Pa.), 0.001% DNase (3,572 U/mg; LSO 2058, Organon Teknika), 5% fetal bovine serum, 100 U of penicillin per ml, and 100 μg of streptomycin per ml. The livers were removed and forced through 50-gauge stainless steel mesh (Cellector; Bellco Glass, Inc., Vineland, N.J.). Cells in the resulting filtrate were washed and then incubated in the solution containing collagenase and DNase for 30 min at 37°C with constant rocking. Nonparenchymal cells were separated from hepatocytes by centrifugation at 3,000 × *g* for 45 min over metrizamide cushions (16% [wt/vol] metrizamide [analytical grade; Nyegaard & Co. AS, Oslo, Norway] in 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES] buffer–6.7 mM KCl–1.22 mM $CaCl_2$ adjusted to a pH of 7.6 with NaOH). After two washes, nonparenchymal cells in RPMI 1640 with 20% fetal bovine serum were plated on 15-mm-diameter glass cover slips. After 1 or 2 h in culture, nonadherent cells were removed by gentle washing. Unless stated otherwise, the remaining, glass-adherent cells were studied immediately.

***P. acnes*.** *P. acnes* (previously termed *Corynebacterium parvum* [11, 31]; Burroughs Wellcome Co., Research Triangle Park, N.C.) was injected intravenously (i.v.) or intraperitoneally (i.p.) at the doses described in Results.

Effect of *P. acnes* on liver histology. Mice were sacrificed 6 days after injection of *P. acnes* i.v. or i.p. Livers were removed, fixed, sectioned, and stained by standard techniques. In some experiments, I prepared a colloidal carbon suspension with 5% India ink in normal saline, passed it

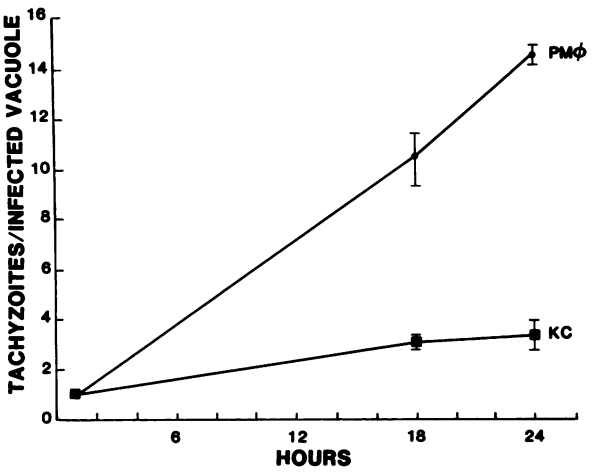

FIG. 1. Multiplication of *T. gondii* within Kupffer cells (KC) and peritoneal macrophages (PMϕ). Bars indicate SD.

through a 0.45-μm (pore size) cellulose filter, and administered 200 μl i.v. 30 min before livers were collected (14).

Peritoneal and spleen macrophages. Peritoneal macrophages were obtained by lavage with a standard technique (7). Spleen macrophages were obtained with a technique that involved mechanical disruption and digestion with collagenase (28).

Phagocytosis of zymosan. Zymosan particles (ICN Pharmaceuticals, Irvine, Calif.) were opsonized in human sera as previously described (29). After they had been allowed to adhere to glass, Kupffer cells were challenged with approximately four particles for every glass-adherent cell. After 1 h, monolayers were washed, fixed, and stained with Giemsa. Numbers of macrophages that had phagocytosed particles were estimated microscopically.

T. gondii. The RH strain of *T. gondii* was maintained in mice as previously described (13). For challenge of macrophage monolayers, tachyzoites were harvested from the peritoneal cavities of mice infected 2 days earlier (1). Tachyzoites were then used to challenge macrophages at a ratio of two tachyzoites per macrophage (1, 8, 14, 15). At intervals, slides were fixed with 0.4% amino acridine in 50% ethanol and stained with Giemsa.

Multiplication of *T. gondii* was assessed microscopically. Numbers of tachyzoites per infected vacuole (1, 8) and numbers of tachyzoites per 100 cells were counted (14, 15). At least 100 infected cells were examined for each slide, and the results for separate slides were averaged. Results were expressed as numbers of tachyzoites per infected vacuole at a particular time (1, 8) or as the change in numbers of tachyzoites per 100 infected cells from 1 h after challenge to the end of the experiment (fold change; 14, 15) as follows: fold change = (tachyzoites per 100 infected cells at end of experiment)/(tachyzoites per 100 infected cells 1 h after challenge). If tachyzoites were killed, the number of tachyzoites per 100 infected cells decreased over time, resulting in a fold change of less than one. If tachyzoites multiplied, the number of tachyzoites per 100 infected cells increased over time, resulting in a fold change of greater than one.

Latent infection with the C56 strain of *T. gondii* was established in mice as previously described (8, 13). Kupffer cells were studied at 3 to 6 months after infection, when the mice appeared well.

Exposure to interferon. Kupffer cells were cultured for 48 h in the presence or absence of recombinant murine gamma interferon (gift of H. Michael Shepard, Genentech, Inc., South San Francisco, Calif.). Media were tested for lipopolysaccharide with the *Limulus* amoebocyte assay as instructed by the manufacturer (Associates of Cape Cod, Inc., Woods Hole, Mass.) and were found to have approximately 0.5 ng/ml. To allow cells to respond adequately to interferon, 3 ng of exogenous lipopolysaccharide (from *Escherichia coli* O111:B4; Sigma Chemical Co., St. Louis, Mo.) per ml was added in some experiments (21).

NBT reduction. Kupffer cells were exposed for 60 min at 37°C to either *T. gondii* tachyzoites or opsonized zymosan particles at a ratio of five particles per Kupffer cell and in the presence of 0.1 mg of Nitro Blue Tetrazolium (NBT; grade III, Sigma) per ml. Cover slips were washed, fixed in methanol, and counterstained with neutral red. Vacuoles containing particles were examined for deep blue-black formazan staining (14).

***G. muris* infection.** *G. muris* cysts, a gift of Stanley Erlandson, University of Minnesota, were passaged monthly in mice. To study the effect of *G. muris* infection on the antimicrobial activities of Kupffer cells, cysts were obtained from fecal slurries by sucrose flotation (24) and quantitated microscopically. One million cysts were introduced by gavage into each uninfected mouse. At 7, 14, 21, and 28 days, Kupffer cells were obtained from livers of infected and control mice, and their antimicrobial activities were measured.

Statistics. Statistical differences between groups of continuous data were tested with the Student *t* test, and those between groups of discontinuous data were tested with the chi-square test. All experiments were performed at least twice.

RESULTS

Characteristics of Kupffer cells. As previously described (20, 22), the procedure to isolate Kupffer cells yielded from 1×10^7 to 2×10^7 cells per mouse liver. Approximately one-third of these adhered to glass after 2 h of incubation. When stained with Giemsa, >99% of these were 10- to 40-μm-diameter mononuclear cells with blue, granular cytoplasm typical of macrophages. The cells resembled adherent cells from peritoneal exudates except that they tended to spread less on the glass surface. More than 99% excluded trypan blue, 99% were esterase positive (33), and 60% phagocytosed zymosan particles. When mice had been injected with colloidal carbon immediately before cells were harvested, 91% of glass-adherent cells contained carbon particles.

Kupffer cells support limited multiplication of *T. gondii*. When challenged with two *T. gondii* tachyzoites per Kupffer cell, 24 ± 6.0% (standard deviation [SD]) of Kupffer cells phagocytosed tachyzoites in 1 h. By 24 h after challenge, Kupffer cells allowed limited multiplication of *T. gondii* (Fig. 1). In four experiments, there was a mean of 3.3 ± 0.5 tachyzoites per infected Kupffer cell vacuole. Peritoneal macrophages challenged in parallel allowed significantly more multiplication: 14.5 ± 0.3 tachyzoites per infected vacuole ($P < 0.001$).

To determine whether differences between Kupffer cells and peritoneal macrophages were attributable to differences in isolation procedures, I exposed peritoneal exudate cells to Hanks balanced salt solution with collagenase and DNase, centrifuged them over a metrizamide cushion, and allowed them to adhere to glass in the same fashion as Kupffer cells. Glass-adherent cells so treated were indistinguishable from control peritoneal macrophages prepared in the usual way.

Effect of *P. acnes* on antimicrobial activity of Kupffer cells. To determine whether administration of heat-killed *P. acnes* affected the ability of Kupffer cells to support the multiplication of *T. gondii*, I injected *P. acnes* i.v. or i.p. In the first 3 days after injection of 175 μg of *P. acnes* i.v., Kupffer cells permitted more multiplication of *T. gondii* than did Kupffer cells from control mice (Fig. 2). During the next 6 to 10 days, there was negligible *T. gondii* multiplication. After 10 days, *T. gondii* multiplication returned to control values.

At 6 days after *P. acnes* injection, when Kupffer cells exerted maximal inhibitory activity, I typically obtained 3.9-fold more interface cells from livers of *P. acnes*-injected mice than from livers of control mice. Similar percentages of interface cells, approximately 30%, from livers of mice that received *P. acnes* and those of control mice were adherent. Macrophages from *P. acnes*-injected mice resembled Kupffer cells from control mice, except that they exhibited greater cytoplasmic spreading on glass. More than 99% excluded trypan blue and 99% were esterase positive.

I compared the ability of liver macrophages from mice injected with 175 μg of *P. acnes* i.v. 6 days earlier to reduce NBT during phagocytosis of *Toxoplasma* tachyzoites or zymosan with the ability of liver macrophages from control, uninjected mice. NBT was reduced in significantly greater proportions of particle-containing vacuoles in macrophages from *P. acnes*-injected mice than in macrophages from control mice (Table 1).

Mice sacrificed 6 days after i.v. injection of 175 μg of *P. acnes* had discrete foci of mononuclear cells in their livers, similar to but smaller than those described in mice infected with *Listeria* sp. (15). In mice injected with colloidal carbon before sacrifice, carbon particles were less abundant in these foci than in surrounding liver, as reported for *Listeria*-infected mice (15). In other parts of these livers and in livers from control mice, carbon particles were found within sinusoids, predominantly in the outer two-thirds of liver lobules as previously described (15).

Macrophages were obtained from livers of *P. acnes*-injected and control mice that were given colloidal carbon immediately before sacrifice. Carbon particles were found in

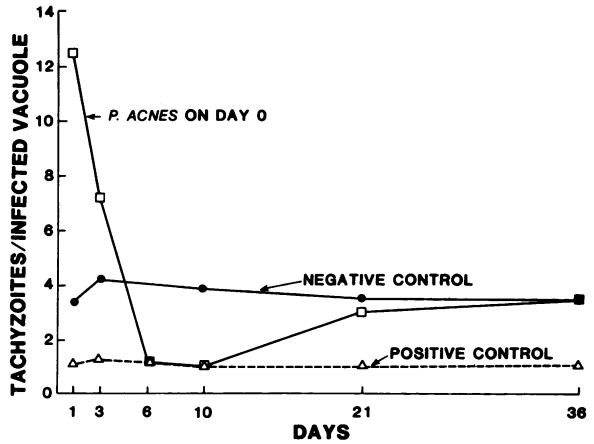

FIG. 2. Effect of i.v. injection of heat-killed *P. acnes* on interactions of liver macrophages and *T. gondii*. Liver macrophages were harvested at intervals (indicated on the abscissa) after injection of *P. acnes* and challenged in vitro (□). At each time point, liver macrophages from unmanipulated mice (negative control [●]) and from mice injected 6 days before the time point (positive control [△]) were studied for comparison.

TABLE 1. NBT reduction by liver and peritoneal macrophages from *P. acnes*-injected and control mice

Macrophages and sources	% (±SD) reduction during phagocytosis of[a]:	
	T. gondii	Zymosan
Kupffer cells from:		
Control mice	7 ± 1	16 ± 6
P. acnes-injected mice	34 ± 18[b]	67 ± 10[b]
Peritoneal cells from:		
Control mice	13 ± 3	57 ± 18
P. acnes-injected mice	31 ± 1	54 ± 11

[a] Percentages of particle-containing cells with reduced NBT are listed. The results represent 10 experiments.

[b] A significantly greater proportion of Kupffer cells from *P. acnes*-injected mice than from control mice reduced NBT after phagocytosis of *T. gondii* ($P < 0.05$) or zymosan ($P < 0.001$).

91% of macrophages from control mice and in 67% of macrophages from *P. acnes*-injected mice. However, the morphologies of macrophages from the two groups of mice were similar. When cultured on glass or plastic for 48 h, fewer macrophages from *P. acnes*-injected mice than from control mice remained adherent. Remaining cells from *P. acnes*-injected mice and controls spread well and had typical macrophage morphology; two distinct subpopulations were not apparent. Cells that could be gently washed off after 48 h in culture did not readhere.

Liver macrophages became more permissive during culture in vitro. After 48 h, multiplication of *T. gondii* within liver macrophages was comparable to that within peritoneal macrophages (Table 2, Kupffer cells cultured for 48 h without interferon).

Effect of recombinant murine gamma interferon. Inclusion of recombinant murine gamma interferon during 48 h of culture maintained the ability of Kupffer cells to inhibit *T. gondii* multiplication partially (Table 2). Kupffer cells incubated with ≥500 U of interferon per ml supported multiplication of *T. gondii* to approximately the same extent as freshly isolated Kupffer cells and significantly less than Kupffer cells incubated without interferon. Concentrations of interferon as high as 50,000 U/ml did not further reduce the ability of Kupffer cells to support the growth of *T. gondii*. When 3 ng of exogenous lipopolysaccharide was added to ensure that cells were able to respond adequately to interferon (21), only 100 U of interferon per ml was required for maximal effects. In contrast, liver macrophages freshly harvested from *P. acnes*-injected mice completely inhibited multiplication. The fold changes of less than one (Table 2) indicated that there was substantial killing of *T. gondii* (14, 15).

To place these results in perspective, I incubated peritoneal macrophages with interferon for similar periods. Doses of interferon that maintained the intermediate ability of Kupffer cells to support the growth of *T. gondii* in culture partially reduced the ability of peritoneal macrophages to support the growth of *T. gondii* in culture (Table 2). Concentrations of interferon as great as 50,000 U/ml did not further reduce the ability of peritoneal macrophages to support the growth of *T. gondii*. In contrast, peritoneal macrophages freshly harvested from *P. acnes*-injected mice exhibited complete inhibition. As with the Kupffer cells, fold changes of less than one (Table 2) indicated that there was substantial killing of *T. gondii*.

Dose response of i.v. *P. acnes*. The effect of i.v. *P. acnes* on interaction of Kupffer cells and *T. gondii* was dose related.

TABLE 2. Effect of recombinant gamma interferon on ability of liver and peritoneal macrophages to support multiplication of *T. gondii*[a]

Time (h) in culture before challenge with *T. gondii*	Liver macrophage mean (±SD)[b]:		Peritoneal macrophage mean (±SD)[c]:	
	Fold change[d]	No. of tachyzoites/vacuole	Fold change[d]	No. of tachyzoites/vacuole
0	2.4 ± 0.3	3.7 ± 0.4	10.7 ± 3.5	12.7 ± 1.1
48 (without interferon)[e]	6.7 ± 1.7	7.7 ± 0.8	11.2 ± 3.1	13.1 ± 0.9
48 (with interferon)[f]	2.1 ± 0.6	4.3 ± 0.6	3.5 ± 2.4	5.5 ± 0.9
0 (*P. acnes*)[g]	0.3 ± 0.1	1.1 ± 0.04	0.3 ± 0.04	1.1 ± 0

[a] The results represent four experiments.
[b] At 24 h after challenge with *T. gondii*.
[c] At 18 h after challenge with *T. gondii*.
[d] Change in average number of tachyzoites per 100 cells over time (from 1 h after challenge to the time point indicated).
[e] Liver macrophages incubated for 48 h without interferon were significantly more permissive than macrophages studied immediately (0 h; $P < 0.05$ for fold change, $P < 0.05$ for number of tachyzoites per vacuole).
[f] Liver macrophages incubated for 48 h with interferon were significantly less permissive than liver macrophages incubated for 48 h without interferon ($P < 0.01$ for fold change, $P < 0.001$ for number of tachyzoites per vacuole).
[g] The macrophages were from mice injected 6 days earlier with *P. acnes* (175 μg i.v. for liver macrophages and 700 μg of *P. acnes* i.p. for peritoneal macrophages).

Mice were injected with *P. acnes* i.v., and their Kupffer cells were harvested 6 days later and challenged with *T. gondii*. Results of three experiments indicated that injection of 44 μg of *P. acnes* or less had no effect, 88 μg partially reduced the ability of Kupffer cells to support multiplication of *T. gondii*, and 175, 350, or 700 μg of *P. acnes* abolished the ability of Kupffer cells to support multiplication of *T. gondii*.

Effect of i.v. *P. acnes* on spleen macrophages. To determine whether *P. acnes* activated all reticuloendothelial system macrophages, I determined the effect of i.v. injection of 175 μg of *P. acnes* on the interactions between spleen macrophages and *T. gondii* 6 days later. Without *P. acnes* injection, spleen macrophages allowed more multiplication of *T. gondii* than did Kupffer cells. In two experiments, there was a mean of 7.5 ± 0.9 (SD) tachyzoites per infected spleen macrophage vacuole after 24 h, compared with 4.6 ± 0.9 tachyzoites per infected Kupffer cell vacuole ($P < 0.001$). As expected, Kupffer cells from mice injected with 175 μg of *P. acnes* 6 days earlier completely inhibited multiplication of *T. gondii*. In contrast, i.v. *P. acnes* injection did not change the ability of spleen macrophages to support the growth of *T. gondii*. Spleens from mice that had received *P. acnes* were congested, but discrete mononuclear infiltrates were not observed.

Effect of i.p. *P. acnes*. Intraperitoneal injection of *P. acnes* also abrogated the ability of Kupffer cells to support multiplication of *T. gondii*, but complete suppression required injection of 700 μg. As reported (17), peritoneal macrophages from such mice also completely inhibited multiplication of *T. gondii*. Livers of mice that received i.p. *P. acnes* had numerous foci of mononuclear cells, which were indistinguishable from those of mice that received i.v. *P. acnes*.

Effect of i.v. *P. acnes* in athymic mice. Kupffer cells obtained from athymic (nude) mice and heterozygous littermates 6 days after i.v. injection of 350 or 700 μg of *P. acnes* completely inhibited multiplication of *T. gondii*. Injection of 175 μg per mouse, a dose sufficient to activate Kupffer cells in BALB/c mice, yielded variable results.

Kupffer cell activity during chronic *Toxoplasma* infection. I established latent infection of mice with the C56 strain of *T. gondii* (17) and confirmed that peritoneal macrophages from mice infected 3 to 6 months earlier allow negligible multiplication of *T. gondii* (25). In contrast, Kupffer cells from such mice allowed moderate multiplication (mean [two experiments], 4.7 ± 0.1 toxoplasmas per vacuole after 24 h), which was indistinguishable from that of Kupffer cells from age-matched controls.

Kupffer cell activity during acute *Giardia* infection. To determine whether Kupffer cell antimicrobial activity might change during acute gastrointestinal infection, I studied Kupffer cells at weekly intervals during and after active infection of mice with *G. muris*. As previously reported (24), *G. muris* trophozoites were consistently detected in fecal samples and small-intestinal washings at 7 and 14 days after infection, inconsistently detected at 21 days after infection, and not detected at 28 days after infection. Cysts were detected throughout this period. In two experiments, multiplication of *T. gondii* within Kupffer cells harvested from such mice at 7, 14, 21, and 28 days after infection with *G. muris* was indistinguishable from that observed in Kupffer cells from control mice.

DISCUSSION

The ability of resident Kupffer cells to support the growth of *T. gondii* was intermediate between that of resident peritoneal macrophages, which are permissive, and that of activated peritoneal macrophages, which are completely inhibitory. Whether the intermediate growth of *T. gondii* within Kupffer cells reflected limited availability of one or more nutrients or the presence of inhibitory substances could not be determined from my experiments.

Since Kupffer cells reside within a solid organ, isolation methods were necessarily more complicated and lengthy than methods for isolation of macrophages from the peritoneal cavity. As reported previously (22), I recovered approximately 3×10^6 Kupffer cells per liver, with excellent viability. Many methods used to harvest Kupffer cells use a nonspecific proteinase mixture to digest hepatocytes. A typical example of these methods has been shown to remove Fc and complement receptor activity from the surfaces of Kupffer cells (6). Although functional receptors reappeared on the surfaces of Kupffer cells prepared with this technique by 48 h in culture (6), I chose instead a method involving the use of collagenase and DNase, which leaves Fc and complement receptors on the surfaces of Kupffer cells intact (22).

There was an interesting biphasic response after i.v. injection of *P. acnes*. During the first 3 days after injection, macrophages harvested from the liver were more permissive for multiplication of *T. gondii* than were macrophages from control mice. From days 6 to 10, macrophages from *P. acnes*-injected mice were highly activated. Later, they returned to baseline activity. A similar biphasic response has been observed in antitumor activity of Kupffer cells after i.v. injection of glucan (26).

The effect of *P. acnes* was dependent on the route of administration in that more heat-killed *P. acnes* was required for maximal activation when it was injected i.p. instead of i.v. The effect of i.v. *P. acnes* was dose dependent, and the lesser potency when it was injected i.p. may mean that less *P. acnes* gained access to the liver. Alternatively, activation of liver macrophages may have resulted from inflammatory mediators which might have reached the liver in lesser amounts after i.p. administration. Systemic administration of *P. acnes* did not activate all macrophages in the body, since the ability of splenic macrophages to support the multiplication of *T. gondii* was not altered.

Injection of *P. acnes* into tumors confers local, tumor-specific immunity, an effect which appears to be dependent on T cells (18). In contrast, systemic antitumor immunity induced by *P. acnes* is T cell independent (32), and direct activation of macrophages by *P. acnes* is not dependent on lymphokines (12). To determine whether T cells were necessary for Kupffer cell activation by *P. acnes*, I administered *P. acnes* i.v. to athymic mice and found that Kupffer cells were activated. The dose required for athymic mice was twofold greater than that required for BALB/c mice, but the same was true for their heterozygous littermates. I concluded that T cells are not required for activation of Kupffer cells by i.v. injection of *P. acnes*.

The effect of systemic infection with the C56 strain of *T. gondii* was somewhat different. As previously reported (17), I found that peritoneal macrophages from such mice were highly activated to kill *T. gondii*. However, interactions of Kupffer cells from such mice with *T. gondii* were indistinguishable from those of Kupffer cells from control mice.

In their position in hepatic sinusoids, Kupffer cells are in contact with blood flowing directly from the gastrointestinal tract. I considered the possibility that the antimicrobial activities of Kupffer cells change during the course of gastrointestinal infection. Although *G. muris* remains localized to the gut during infection, I hypothesized that microbial products or inflammatory mediators from the local intestinal immune response might alter the antimicrobial activities of Kupffer cells. My hypothesis appeared to be incorrect, because the interactions of Kupffer cells and *T. gondii* remained unchanged throughout active *G. muris* infection.

My results are in keeping with those in previous reports which indicate that Kupffer cells are able to produce reactive oxygen intermediates in vitro (2, 3, 9, 16) and that this ability can be enhanced by systemic inflammatory stimuli (2, 9, 10, 16). I found that macrophages harvested from livers 6 days after *P. acnes* injection reduced NBT to a much greater extent than did control macrophages, indicating that they were better able to form reactive oxygen intermediates within their vacuoles.

My results differ from observations of murine liver macrophages by Lepay et al. (14, 15). They found that cells from unmanipulated mice were as permissive for *T. gondii* as were peritoneal macrophages. This was reflected by inactive oxidative metabolism; for example, liver macrophages reduced little NBT during phagocytosis of zymosan or *Leishmania* promastigotes. During sublethal *Listeria* infection, livers had discrete foci of mononuclear cells. In livers from mice injected with colloidal carbon immediately before sacrifice, the carbon was less abundant in these foci than in other parts of the livers. Macrophages from livers of *Listeria*-infected mice appeared heterogeneous, with two discrete populations. One population resembled macrophages from uninfected mice, contained abundant carbon particles, and did not become inhibitory for *T. gondii* or produce reactive oxygen intermediates. The other population, which infiltrated the liver in response to *Listeria* infection, was morphologically different and had fewer carbon particles, which suggested they had been in the foci of mononuclear cells in vivo. They could be removed with gentle washing, but they readily readhered. They produced large amounts of reactive oxygen products in response to stimuli. Antimicrobial properties were not evaluated because these macrophages did not phagocytose *T. gondii* in culture.

In my experiments, several observations suggested that the entire population of macrophages harvested from livers 6 days after *P. acnes* injection was activated. Like Lepay et al. (15), I observed foci of mononuclear cells in livers of *P. acnes*-treated mice, and fewer macrophages from such mice contained carbon particles. However, I did not observe two morphologically distinct populations of macrophages in cultures from such mice. Most importantly, our assay for antimicrobial activity involved examination of individually infected cells for growth of *T. gondii*. The existence of a permissive subset of cells would have been apparent as a subset with marked multiplication of tachyzoites. Such a subset was not observed.

I did not observe a loosely adherent subpopulation of macrophages from *P. acnes*-injected mice after 48 h in culture which would readhere on a fresh surface. What I learned was that the innate resistance to multiplication of *T. gondii* within Kupffer cells was lost during 48 h of culture in vitro. This may explain part of the differences between the results of Lepay et al. and my own.

Lepay et al. found that liver macrophages did not develop increased antimicrobial activity or increased activity of their oxidative metabolic bursts in the presence of recombinant gamma interferon (14). In contrast, I found that recombinant gamma interferon obtained from the same source maintained the relative inhibitory activity of my Kupffer cells. The activities of these Kupffer cells were similar to those of peritoneal macrophages incubated with interferon.

Neither Kupffer cells nor peritoneal macrophages became completely inhibitory in the presence of interferon. This did not seem to be due to inadequate exposure to interferon, because the effect reached a plateau with intermediate doses. Since both liver and peritoneal macrophages are capable of complete inhibition in vivo, the results implied either that interferon is more active in vivo or that other mediators (19) contribute to the highly activated states of these cells during inflammatory responses in vivo. Several previous observations have also indicated that the process of macrophage activation is complex. Some investigators have found that antimicrobial and antitumor properties of activated macrophages dissociate under some conditions (4, 30), and others have found dissociation between killing of different kinds of microorganisms and oxidative metabolism (27).

The differences between the results of Lepay et al. (14, 15) and my own cannot be completely explained. There were differences in mouse strains, methods of isolation and culture of Kupffer cells, the inflammatory stimuli (*Listeria* infection and heat-killed *P. acnes* injection), and the interval between isolation of Kupffer cells and challenge. It is clear from the observations of Lepay et al. that, under some conditions, there are two distinct populations of liver macrophages with markedly different properties. It is clear from my results that, under other conditions, Kupffer cells can be activated in vivo and can respond to interferon in vitro to

increase, or at least maintain, their moderate antimicrobial activity.

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