Tuberculin Hypersensitivity Hepatitis in Mice Infected with Mycobacterium bovis *(BCG)*

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A systemic BCG infection in mice induced multiple small granulomas located mainly in the periportal areas of the liver. Following systemic challenge of such mice with purified protein derivative of tuberculin (PPD), a rapidly developing hepatitis with diffuse intralobular mononuclear cell infiltration was precipitated, accompanied by high levels of aspartate transaminase in peripheral blood, hypoglycemia, focal hepatocyte necrosis, and accumulation of fibrinogen in liver. Bacterial lipopolysaccharide (LPS) also provoked acute hepatic damage both in BCG-infected mice and in mice pretreated with *Corynebacterium parvum*. PPD was not active in the latter. There were also lymphoid cell destruction and fibrinogen accumulation in the spleen of BCG-PPD–treated mice. Possible involvement of inflammatory and hepatotoxic mediators is suggested, and a T-lymphocyte–macrophage regulatory role in the pathogenesis of hepatitis is discussed. (Am J Pathol 1981, 105:82–90)

SEVERAL STUDIES have shown that parasitic, bacterial, or viral infections, such as mouse hepatitis virus,[1] *Mycobacterium bovis* (BCG),[2] *Salmonella typhosa*,[2] or *Schistosoma mansoni*[3] infection, render experimental animals very susceptible to the lethal effect of bacterial endotoxins or their purified lipopolysaccharide preparations (LPS). There is a general stimulation of the mononuclear phagocytic cell system, often accompanied by induction of multiple granulomas in the liver of infected animals. In mice infected systemically with BCG and later challenged with a minute amount of LPS, Shands and Senterfitt[4] found an acute and extensive liver parenchymal cell injury, detected by microscopic examination and by high levels of liver enzymes in blood.

One possible interpretation of the pathogenesis of the liver damage would be that macrophages recruited into the liver by an infectious agent release large amounts of hepatotoxic soluble mediators following the second stimulus such as LPS. To test further this hypothetical two-stage model, an acute hypersensitivity hepatitis was produced by systemic injection of the antigen purified protein derivative of tuberculin (PPD) into BCG-infected mice. This procedure produced a rapid diffuse infiltration of liver lobules with mononuclear inflammatory cells, accompanied by hepatocyte damage. The effects of LPS, the second, immunologically nonspecific stimulus, were compared in BCG-infected mice and in mice made "hyperreactive" by an intravenous injection of killed *Corynebacterium parvum*.[5]

Materials and Methods

Animals

Female CBA mice, aged 10–15 weeks, bred in specific pathogen-free conditions at the Clinical Research Centre, Harrow, Middlesex, U.K., were used.

BCG Infection and *Corynebacterium parvum*, PPD, and LPS Injections

M bovis (BCG), grown for 14 days in a glycerol-free culture medium, was kindly donated by Glaxo Lab. Ltd, Greenford, Middlesex, U.K. Mice were injected via a tail vein with 0.2 ml of the BCG culture (approximately 2×10^7 viable units), with the culture medium alone, or with 0.7 mg/mouse of killed *C parvum* (Wellcome Reagent Ltd., Beckenham, U.K.) suspended in 0.2 ml of saline. Mice treated 10 days earlier with BCG, mice treated 7 days earlier with *C*

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parvum, and normal mice, were injected intravenously with 1 mg/mouse of purified protein derivative of human tuberculin (PPD, batch 298, a gift from Central Veterinary Lab., Weybridge, Surrey, U.K.), or intravenously with 10 μg/mouse of the lipopolysaccharide B (LPS, *Escherichia coli*, 055:B5, Difco Lab., Detroit, Mich) dissolved in 0.2 ml of saline, or with saline alone. After various time intervals, mice were exsanguinated under ether anaesthesia from the axillary blood vessels, and blood and tissue samples from various organs were collected.

Blood Aspartate Transminase and Glucose Estimation

Part of the blood was collected into tubes containing 20 IU of heparin/ml of blood, and, after sedimentation of cells, plasma was examined for aspartate transaminase activity by the method of Karmen.[6] The remainder of the blood was added to tubes containing 10 mg/ml of sodium fluoride, and the serum was analyzed for glucose content by the glucose oxidase method of Trinder.[7]

Histologic Studies

Slices of liver, spleen, lungs, and kidneys were fixed in 10% neutral formalin and embedded in paraffin wax. The sections (4 μ) were stained with hematoxylin and eosin or by the MSB method for fibrin.

Footpad Tests

Ten days after BCG infection, mice were injected at the right hind footpads with 5, 30, or 50 μg of PPD in 0.05 ml of saline and at the left footpads with the 0.05 mg of saline alone. The thickness of the footpads was measured with a calipers-micrometer after 6 and 24 hours, and the percentage of increase in thickness of the PPD-injected footpads as compared with the saline-injected footpads was calculated as described by Salvin et al.[8]

Labeled Fibrinogen and Albumin Distribution

Iodinated (^{125}I) human fibrinogen (injection) and iodinated (^{131}I) human serum albumin (injection BP) were obtained from the Radiochemical Centre, Amersham, U.K. BCG-infected mice and normal mice were injected intravenously with a mixture of 1 μCi ^{125}I-fibrinogen and 1 μCi ^{131}I-albumin per mouse in 0.2 ml of saline containing 1% of an unlabeled human serum albumin as carrier. This treatment was followed 4–6 hours later by intravenous injections of PPD, LPS, or saline alone. After various time intervals, blood and organ samples were collected and weighed, and their ^{125}I and ^{131}I radioactivities were measured in a LKB Wallac 1280 gamma counter (LKB, Broma, Sweden). The percentage of radioactivity of organ relative to that of blood was calculated for individual mice. For evaluation of fibrinogen degradation, blood samples were collected in tubes containing 5 NIH units/ml of bovine thrombin (Parke-Davis, Pontypool, U.K.) and a 1% final concentration of ε-aminocaproic acid to facilitate coagulation and to prevent subsequent fibrinolysis. The percentage of ^{125}I-fibrinogen that was unable to clot was calculated by comparing the radioactivity remaining in the serum with that in whole blood, on the basis of weight.

Expression of Results

Mean values, usually obtained from 3 mice per group, and SD are indicated.

Results

Rise of Aspartate Transaminase Levels in Blood on PPD Challenge of BCG-Infected Mice

Mice were infected with 2×10^7 BCG intravenously and, after various time intervals, challenged intravenously with 1.0 mg PPD. Usually 10 hours later, the elevation of the liver enzyme marker aspartate transaminase levels in blood was taken as a measure of hepatocyte damage. There was a marked increase in aspartate transaminase levels 10–12 days after the infection reaching 1400 IU/l plasma (Table 1). At this stage of the infection, the background enzyme level (ie, the level without PPD) was raised from about 150 IU/l plasma in normal mice to about 500 IU/l plasma

Table 1—Development of Blood Aspartate Transaminase Response to PPD in Mice After BCG Infection*

Days after BCG infection	Aspartate transaminase (IU/1 plasma)	
	PPD	Saline
5	188 ± 50	ND
7	185 ± 10	ND
10	1439 ± 128	685 ± 61
12	1040 ± 57	634 ± 11
14	478 ± 214	174 ± 13
16	390 ± 47	269 ± 34
19	253 ± 14	154 ± 11
22	206 ± 8	162 ± 14
30	213 ± 33	164 ± 21
60	152 ± 40	ND
Uninfected mice	159 ± 39	165 ± 16

* Blood samples taken 10–11 hours after challenging the mice intravenously with 1 mg PPD or saline.
ND = not determined.

in BCG-infected mice, which suggested a hepatocyte injury caused to some extent by the infection alone (Table 1 and 2). In the BCG-infected mice, the aspartate transaminase level rose further to a plateau level of about 1300 IU/l plasma 4 hours after challenge with PPD. Such mice usually showed only transient shock and recovered after 24 hours. Doses lower than 1 mg of PPD raised the enzyme levels irregularly, as tested in separate experiments in 10–11-day BCG-infected mice within 8–10 hours: 0.3 mg PPD, 1243 ± 663, 1162 ± 390 IU/l plasma; 0.1 mg PPD, 1064 ± 104, 1296 ± 248, 493 ± 117, 607 ± 31 IU/l plasma. The enzyme levels in saline-injected mice ranged from 300 to 685 IU/l plasma.

Footpad Tests with PPD in BCG-Infected Mice

Injections of 5 μg or 30 μg of PPD into right hind footpads 10 days after BCG infection produced 0.7 ± 1.4% and 2.6 ± 2.3% increases in thickness, respectively, after 6 hours and 1.5 ± 1.7% and 14.9 ± 1.8% increases in thickness, respectively, after 24 hours relative to left footpads injected with 0.05 ml of saline alone. In another experiment, on injection of 50 μg of PPD, there was only a 10.8 ± 1.8% increase in footpad thickness in 24 hours.

Comparison of the Aspartate Transminase and Glucose Content Changes in Blood Between BCG- and *C parvum*-Treated Mice

As a control for immunologic specificity, mice made hyperreactive to LPS by pretreatment with killed *C parvum* were also challenged with PPD (Figure 1). In such mice, PPD did not raise aspartate transminase levels or lower the glucose content in blood, whereas LPS did so. Mice were usually tested 7 days after *C parvum* treatment because of their high susceptibility to LPS at that time, but the results at 11 days were similar. In contrast to PPD-treated mice (Table 1), mice treated with 10 μg of LPS 7 days after the BCG-infection responded with about a 1400-IU/l rise in plasma aspartate transaminase activity. LPS in this dose was often lethal (in about 70% of cases) in both *C parvum* and BCG-pretreated mice, but not in normal mice.

Table 2—Time Course of Elevation of Blood Aspartate Transaminase Levels in Mice Infected with BCG and Challenged with PPD*

Hours after administration	Aspartate transaminase (IU/1 plasma) PPD	Saline
2	734 ± 14	
4	1226 ± 146	527 ± 40
6	1270 ± 152	
8	1402 ± 325	468 ± 42
10	1356 ± 97	
20	1274 ± 162	474 ± 40
48	570 ± 46	522 ± 19

* Mice infected intravenously with 2 × 10^7 BCG and 10 days later injected intravenously with 1 mg PPD or saline only.

^{125}I-Fibrinogen Distribution in Various Organs from BCG-PPD-Treated Mice

To obtain an indication of fibrin deposition at the site of inflammation, BCG-infected mice were injected intravenously with ^{125}-I-labeled fibrinogen prior to challenge with 1 mg of PPD. 4 hours later, but to a greater extent 6½ hours later (Table 3), there was an accumulation of ^{125}I-fibrinogen in liver and spleen exceeding the accumulation of ^{131}I-albumin. This level increased further after 24 hours. LPS was much more effective, with ^{125}I-fibrinogen content in spleen surpassing that in the blood. However, the infection alone induced a moderate accumulation of fibrinogen in infected mice as compared with normal

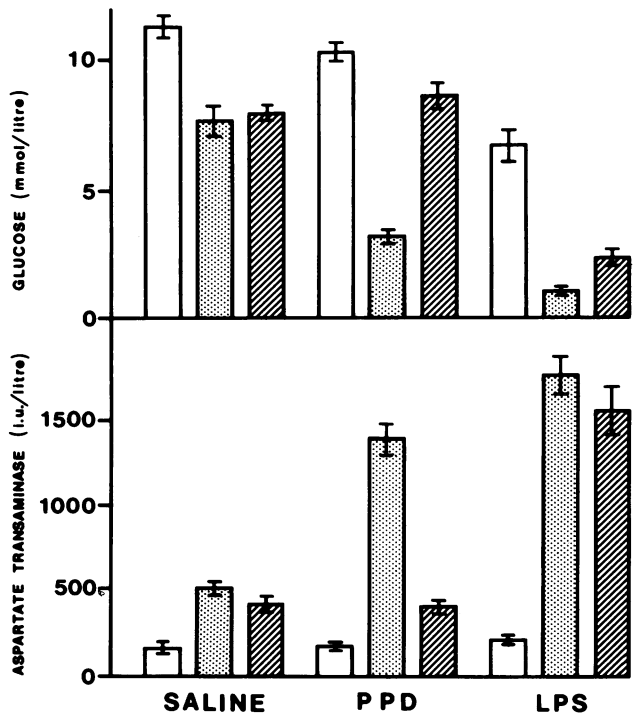

Figure 1—The effect of PPD and LPS on blood glucose and aspartate transaminase levels in mice. Normal mice (*open columns*), mice infected intravenously with 2 × 10^7 BCG 10 days earlier (*dotted columns*), and mice injected intravenously with 0.7 mg of killed *C parvum* 7 days earlier (*hatched-columns*) are then challenged intravenously with 0.2 ml saline, 1 mg of PPD, or 10 μg of LPS. Blood samples taken 6–7 hours later. Mean values and SD from 4 mice per group.

Table 3—Effect of PPD and LPS on the Distribution of ^{125}I-Fibrinogen and ^{131}I-Albumin in BCG-Infected Mice*

Treatment	Percent label:tissue/blood			
	Liver	Spleen	Lung	Kidney
Saline				
^{125}I	20.7 ± 0.2	30.7 ± 1.9	13.3 ± 0.9	22.9 ± 4.4
	(25.7 ± 2.0)	(39.1 ± 2.8)	(28.6 ± 2.8)	(22.8 ± 1.2)
^{131}I	13.1 ± 0.2	21.7 ± 0.2	28.6 ± 3.6	21.9 ± 4.4
	(16.9 ± 0.9)	(23.9 ± 1.3)	(39.7 ± 0.5)	(25.5 ± 2.1)
PPD				
^{125}I	30.7 ± 1.7	43.0 ± 2.7	18.6 ± 1.6	27.6 ± 6.2
	(52.0 ± 3.4)	(77.1 ± 7.0)	(39.9 ± 1.7)	(30.6 ± 1.5)
^{131}I	16.0 ± 0.3	22.6 ± 1.1	34.2 ± 2.1	25.4 ± 2.0
	(19.2 ± 0.4)	(25.6 ± 1.4)	(40.5 ± 2.2)	(29.4 ± 0.8)
LPS				
^{125}I	80.4 ± 5.8	132.7 ± 35.6	18.2 ± 2.1	31.1 ± 1.4
^{131}I	15.7 ± 0.6	26.2 ± 2.4	36.2 ± 3.2	24.1 ± 1.5

* Ten days after the BCG infection, mice were injected intravenously with 1 μCi of each labeled protein, and 4 hours later, they were injected intravenously with saline, 1 mg of PPD, or 10 μg of LPS. Blood and organ samples were collected 6½ hours and 24 hours (numbers in parentheses) after PPD or LPS challenge. Mean values and SD were from 3 mice, except with LPS treatment, from 5 mice per group.

mice. In normal mice, the liver/blood percentage distributions for ^{125}I-fibrinogen and ^{131}I-albumin, respectively, were 14.1 ± 2.6%, and 14.0 ± 1.1% 7 hours after saline injection, and 13.9 ± 0.5% and 13.8 ± 0.8% with 1 mg of PPD, and 17.0 ± 1.7% and 15.6 ± 0.9% with 10 μg of LPS. The BCG infection and PPD challenge experiment was repeated twice with similar results. After 20 hours, a 0.5-mg dose of PPD induced a 47.0 ± 9.3% liver/blood distribution of ^{125}I-fibrinogen compared with 21.7 ± 0.8% in saline control mice. Values for ^{131}I-albumin were 19.6 ± 0.7% and 18.9 ± 1.2%, respectively. A 0.1-mg dose of PPD was not effective.

To assess whether fibrinogen-fibrin degradation had occurred during the reaction to PPD, the amount of ^{125}I-fibrinogen that was unable to clot in serum was calculated. Six and a half hours after challenge, the serum/blood percentages of noncoagulatable ^{125}I-fibrinogen in mice injected with saline and 1 mg of PPD, respectively, were 6.9 ± 1.4% and 19.0 ± 1.3% in BCG-infected mice, and 3.6 ± 0.5% and 3.8 ± 1.4% in uninfected mice.

Histologic Findings in Liver and Spleen

Morphologic alterations in liver of BCG-infected and PPD-challenged mice roughly paralleled the elevation of the aspartate transminase levels in blood. Figure 2 shows multiple granulomas consisting of mononuclear cells that developed during 10 days of BCG infection. These granulomas tended to be localized mainly, but not exclusively, in the periportal areas. Four and a half hours after BCG-infected mice were challenged intravenously with 1 mg of PPD, there was a diffuse infiltration of liver lobules with predominantly mononuclear inflammatory cells, which thus obscured the boundaries of the granulomas and made them less apparent (Figure 3). Liver parenchyma appeared compact and the sinusoids appeared compressed, so that the normal radial architecture of lobules was obscured. This may have

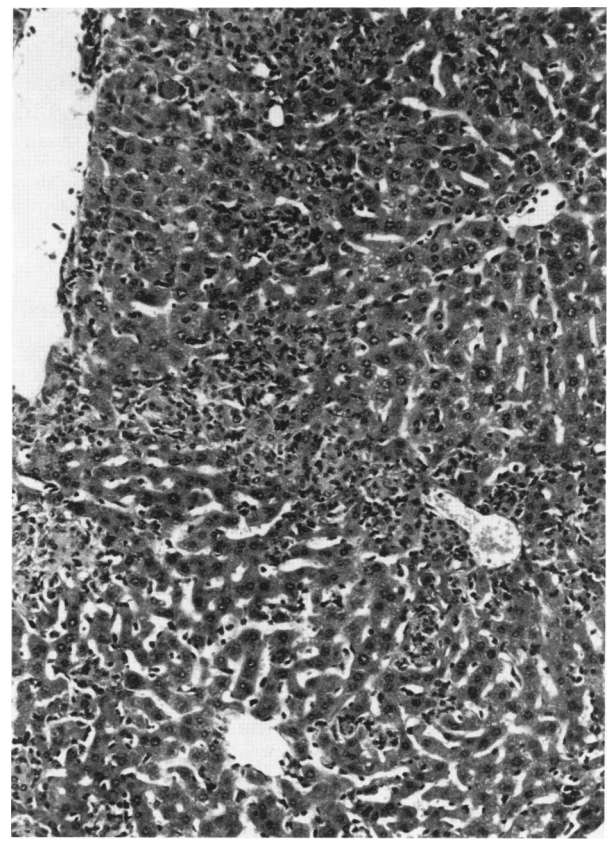

Figure 2—Liver from a mouse 10 days after the intravenous infection with 2 × 10^7 BCG and 4½ hours after an intravenous injection of 0.2-ml saline only. There are multiple, mainly periportally located, mononuclear cell granulomas with otherwise preserved lobular structure. (H&E, × 180) (With a photographic reduction of 27%)

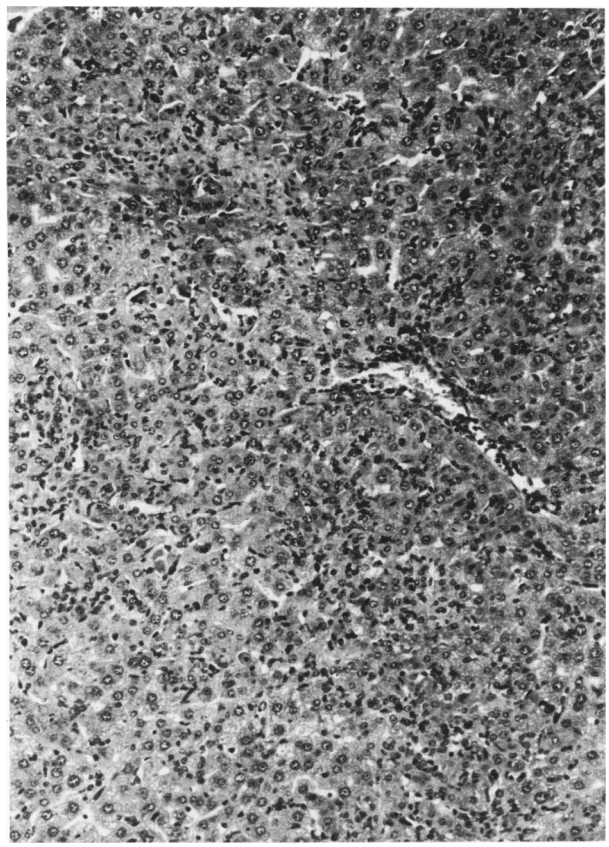

Figure 3—Liver from a mouse treated in the same way as in Figure 2, except for an intravenous injection of 1 mg of PPD instead of saline 4½ hours before sacrifice of the animal. Inflammatory cell infiltration appears more diffuse and the lobular cordlike architecture is no longer distinguishable. (H&E, × 180) (With a photographic reduction of 27%)

been due to the presence of the inflammatory cells, but is more plausibly attributed to swelling of hepatocytes. In addition, about 6 hours later, focal areas of hepatocyte necrosis could be seen (Figure 4). One day after PPD challenge, necrotic cell foci were more pronounced (Figure 5). At that time, such lesions were heavily infiltrated with scavenging mononuclear cells, which presumably were newly emmigrated. Hepatocytes appeared grossly vacuolated throughout the liver section. Two days after PPD administration, the diffuse inflammatory cells began to clear, and by the third or fourth day liver lobules had assumed a normal appearance, except for the persistance of the discrete granulomas found before PPD challenge. Occasional mitotic figures in hepatocytes could be seen at that time. In mice examined 24 hours after PPD injection, the fibrin deposition in liver suggested by the isotope studies could not be seen by the MSB staining method.

Livers from 41 mice with a 10–11-day BCG infection and challenge with 1 mg of PPD were examined in 12 experiments and compared with an equal number of livers from BCG-infected and saline-injected control mice. The intensity of liver changes varied individually and the changes were not uniformly distributed in the liver sections. Usually, about one third to two thirds of the lobuli in a lobe was as severely affected as that shown in Figure 3. A 0.3-mg dose of PPD caused less obvious changes, and with a 0.1-mg dose of PPD only a moderate vacuolation and a few degenerating hepatocytes were seen after 24 hours in 6 out of 13 animals.

When mice were challenged with PPD 16 days after BCG infection, no diffuse cellular infiltration of liver or hepatocyte changes could be seen, in spite of the presence of granulomas that were assuming a circumscribed concentric epithelioid appearance (Figure 6). Twenty to 30 days after the infection, the granulomas were fewer in number and smaller and were composed mainly of epithelioid cells. LPS (10 μg) induced similar liver changes in BCG-infected mice.

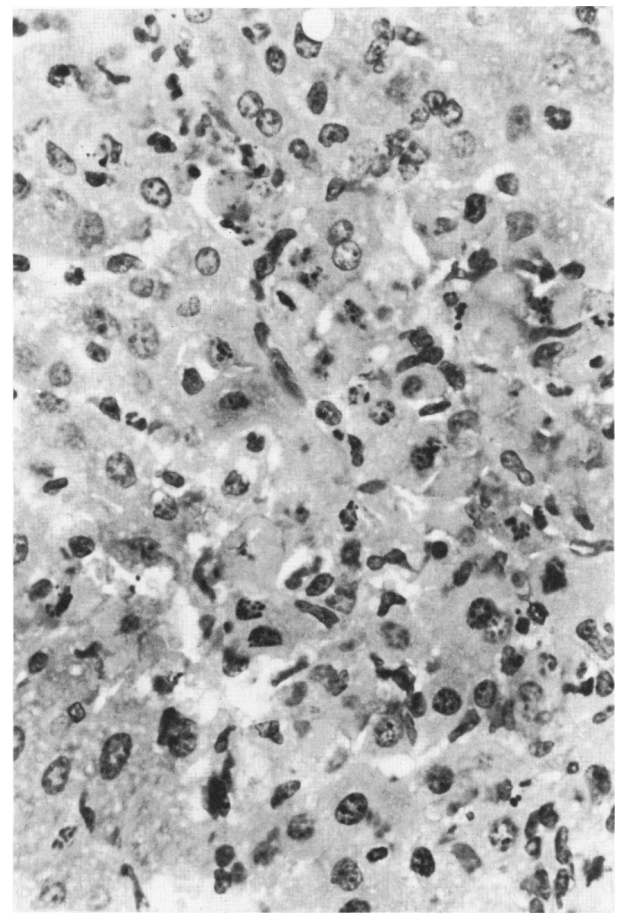

Figure 4—Liver from a 10-day BCG-infected mouse 6½ hours after PPD challenge, showing focal necrosis of hepatocytes with eosinophilic cytoplasm and hyperchromatic pyknotic and fragmented nuclei. (H&E, × 745) (With a photographic reduction of 21%)

In spleen, the BCG infection induced a lymphoid cell hyperplasia mainly in the red pulp, and PPD challenge caused lymphoid cell degeneration already detectable at 6 hours and more pronounced at 24 hours (Figure 7). LPS (10 μg) produced similar changes in spleen of such mice. In lungs, a 10-day BCG infection produced a moderate interstitial infiltration of mononuclear leukocytes with a slight proliferation of fibroblasts. On PPD challenge of mice, there was only a slight additional inflammatory cell infiltration, but no obvious exudation into alveolar spaces. In kidneys, no apparent changes could be seen during BCG infection or after PPD challenge.

C parvum treatment of mice also induced granulomas in liver and hyperplasia of spleen. In such mice, 10 μg of LPS provoked similar changes in lobular structure with focal hepatocyte necrosis, but the inflammatory cell infiltration was generally somewhat less extensive than in BCG-PPD–treated mice. Challenge with 1 mg of PPD was apparently not effective. Lymphoid cell degeneration in spleen of LPS-challenged mice was also observed. In normal mice or mice injected 10 days earlier with the glycerol-free culture medium alone, there were no apparent alterations in liver and spleen after challenging with 1 mg of PPD or 10 μg of LPS.

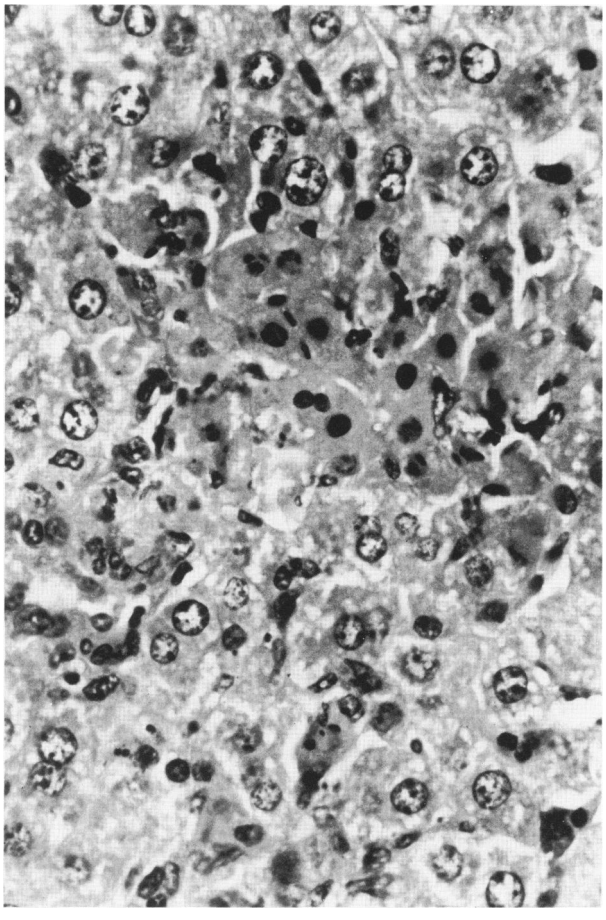

Figure 5—Liver obtained 24 hours after PPD challenge from a 10-day BCG-infected mouse with a focal hepatocyte necrosis and a generalized vacuolation of the cells. (H&E, × 745) (With a photographic reduction of 21%)

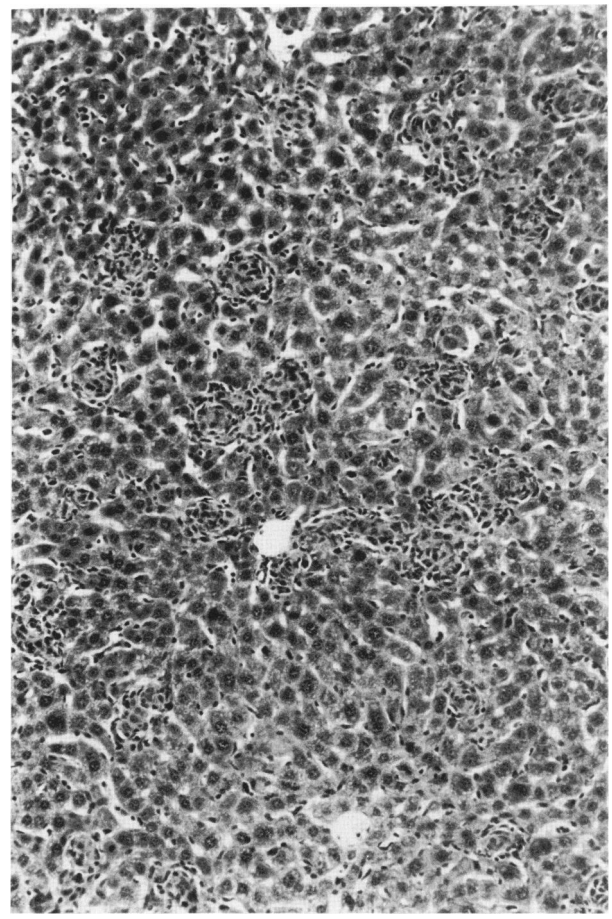

Figure 6—Liver from a mouse infected with BCG 16 days earlier and 8 hours after PPD challenge, showing the circumscribed granulomas with absence of an acute diffuse inflammation. (H&E, × 180) (With a photographic reduction of 21%)

Discussion

A systemic BCG-infection in mice induced liver granulomas located predominantly periportally. Such mice reacted to a challenge with PPD with an acute diffuse intralobular mononuclear cell hepatitis, accompanied by damage to parenchymal cells. One reason for an accelerated peak tuberculin response in liver at around 6 hours, as compared with a delayed response in skin,[9] may be that sensitized lymphocytes and auxiliary cells, such as macrophages, are already locally accumulated in granulomas. It is postulated that, when stimulated by the antigen PPD, T lymphocytes release chemotactic, immobilizing factors

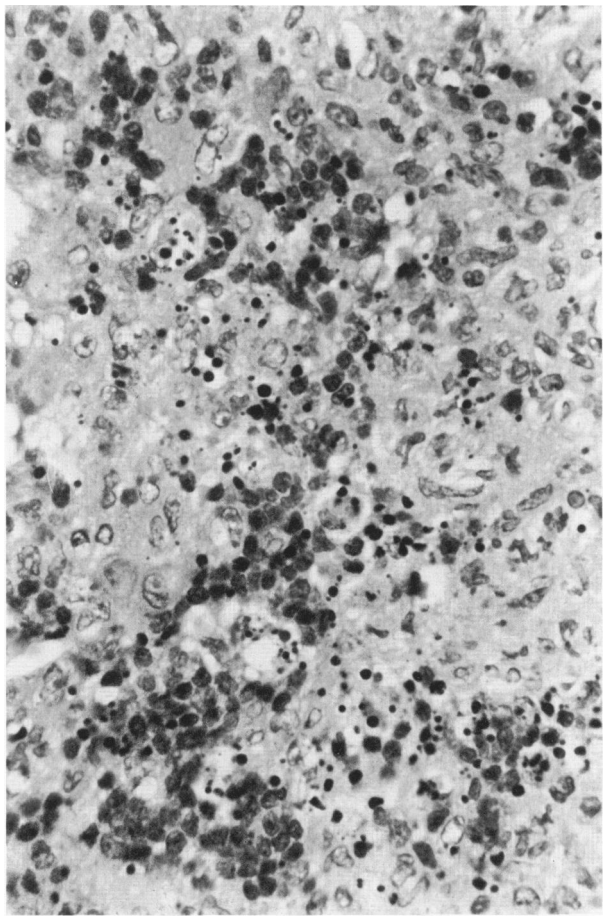

Figure 7—Spleen section from a mouse 10 days after BCG infection and 24 hours after PPD challenge, demonstrating necrosis of lymphoid cells with hyperchromatic fragmented nuclei. (H&E, ×745) (With a photographic reduction of 21%)

(MIF) and activating factors (MAF, interferon) for macrophages [10,11] and natural killer cells,[12,13] which would then diffuse from the periportal region into liver sinusoids. Here, additional inflammatory cells from the blood would be attracted and activated. This assumption derives support from the discovery of high levels of MIF and interferon in blood of similarly treated mice.[8,14]

Hepatocytes may be damaged as "bystanders" by the inflammatory cells on contact, or by their potentially cytotoxic products, such as hydrolytic enzymes,[4,15] oxidative prostaglandin intermediates,[16] and hydrogen peroxide.[17] Kupffer cells may also become activated and hepatotoxic.[18] Liver sinusoidal endothelial cells are discontinuous and fenestrated and would thus facilitate the passage of inflammatory cells and solutes into the space of Disse.[19] Inhibition of gluconeogenesis, mediated by macrophage "glucocorticoid antagonizing factor" has been implicated as the main cause of hypoglycemia in BCG-LPS-treated mice.[20,21] In PPD-challenged mice, blood glucose levels were not as low, which may explain the survival of the majority of these animals.

Accumulation of fibrinogen at a delayed hypersensitivity inflammation site was found previously[22] and is probably due to coagulation, triggered by macrophage-produced thromboplastin.[23] It has been suggested that MIF inhibits leukocyte migration by this process.[24] The potential blood coagulation in liver sinusoids, with a resulting hypoxia, probably did not contribute substantially to hepatocyte damage initially, since the increase in the labeled fibrinogen in liver was not high within 6 hours after PPD injection in BCG-infected mice. It is also possible that a degradation of fibrin was taking place through the action of macrophage plasminogen activator.[15,23] An increase in noncoagulatable ^{125}-I-fibrinogen in blood was suggestive of fibrinogen turnover.

Since PPD-elicited hepatitis appeared antigenically specific, it is unlikely that it was caused by endotoxin contamination of the PPD. A small amount of LPS in *C parvum*-pretreated mice produced similar hepatic changes, whereas PPD did not. Dextran sulphate and zymosan also elicited gross liver damage in BCG-and *C parvum*-treated mice (unpublished data). Such inflammatory agents, and immune complexes as well, probably stimulate macrophages via the activation of complement.[23] Necrosis of lymphatic tissue, as seen in the spleen, appears to be a general feature of some infections, such as malaria[25] and theileriosis of cattle, and is possibly attributable to natural killer cells.[26]

The timing of the hepatitis response to PPD coincided with the skin responses previously reported by Rook.[27] The 10-day reaction is thought to reflect immune protection against *Listeria*-type intracellular parasites. Possibly, the mice were mounting such a response at that time to overcome the infection, and they apparently did so successfully as judged by the subsequent regression of granulomas. Activated granuloma macrophages that are generally able to kill the bacteria intracellularly[28] may have been damaging to the nearby hepatocytes. This result was seen previously[4] and is indicated by the elevated background transaminase blood levels. It is probably only with excessive amounts of free antigen that a diffuse hepatitis would develop. High doses of PPD needed to elicit this response and a footpad reaction suggest that the mice were already immunosuppressed to some degree at this stage. This type of response has been shown to be regulated by a feedback inhibition of production of T-lymphocyte factors, brought about by macrophage-produced E-prostaglandins.[29,30] This process would restrict tissue injury

but perhaps also diminish the immunity. The low responsiveness to PPD in tuberculous patients has been ascribed partly to a monocyte-effected inhibition,[31] and mice are likely to be affected in the same way. A hypersensitivity hepatitis to oxazolone could be induced in mice provided that their suppressor cells had been inactivated.[32]

The even lower responsiveness after 12 days of infection may be due to macrophage or to suppressor lymphocyte-mediated inhibition,[27] or to maturation of granuloma monocytes. The latter became progressively more epithelioid. Only the newly engaged inflammatory macrophages could be stimulated by T-cell products to become cytotoxic,[33] or by LPS to release a lysosomal hydrolase.[34] The recruitment of monocytes from the blood decreased probably because the BCG infection did not persist.

In viral B hepatitis a cell-mediated immunity component also seems to be involved.[35] This involvement may be viewed as an imbalanced delayed hypersensitivity reaction. There may be an accumulation of inflammatory cells periportally, which is followed, in cases with excessive viral antigens or a diminished immunosuppression,[36] by a clinically overt diffuse hepatitis. Similar immunopathogenetic mechanisms may operate in other infections, such as malaria[37] and schistosomiasis, where parasitaemic waves or endotoxins from gut flora may provoke episodes of acute hepatitis.

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