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Protective Immunity and Granuloma Formation Are Mediated by Two Distinct Tumor Necrosis Factor Alpha- and Gamma Interferon-Dependent T Cell-Phagocyte Interactions in Murine Listeriosis: Dissociation on the Basis of Phagocyte Adhesion Mechanisms

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Listeria-immune mice are able to express protective immunity in the absence of CD4$^+$ T cells and an apparent granulomatous inflammation. Using a monoclonal antibody (5C6) able to inhibit the recruitment of myelomonocytic cells into inflammatory foci by binding to complement receptor type 3 (CR3/CD11b), we could show that protective immunity and granuloma formation indeed depend on two distinct types of T cell-phagocyte interactions. Listeria-specific CD8$^+$ T lymphocytes, possibly in collaboration with CD4$^-$ CD8$^-$ T cells, rapidly interact with myelomonocytic cells infiltrating infected tissues in a CR3/CD11b-dependent manner. This interaction results in potent antilisterial protection but not in granuloma formation. On the contrary, CD4$^+$ T cells are able to induce adhesion mechanisms that allow the accumulation of monocytes in granulomatous lesions even in the presence of monoclonal antibody 5C6. However, the protective capacity of these CR3/CD11b-independent T cell-mediated immune mechanisms is low in listeriosis. Tumor necrosis factor alpha and gamma interferon, known to be essential for the expression of both resistance and acquired immunity, are shown to be necessarily involved in granuloma formation, too. It therefore remains to be explained why CD8$^+$ T cells, able to secrete both cytokines, do not induce granuloma formation. The data point to the presence of an as yet undefined CD4$^+$ T cell-derived granuloma-inducing factor and favor the hypothesis that CD8$^+$ T cells, in collaboration with circulating phagocytes, mediate immunity by rapidly liberating listeriae from permissive cells or protecting them from becoming infected.

The adequate function of sensitized T lymphocytes is essential for both protective immunity and granuloma formation in response to intracellular pathogens. While the CD4$^+$ subset in most cases mediates the inflammatory phenomena, CD8$^+$ T cells are crucially involved in protection against facultative intracellular bacteria (26), fungi (23), and parasites (39, 57). In addition, very recently, protective CD4$^-$ CD8$^-$ T cells have been demonstrated in the listeriosis model (14, 22). However, the effector mechanisms operative in the expression of acquired immunity in the absence of CD4$^+$ T cells are not clear. As previously shown (30, 32, 35, 45), listeria-primed CD8$^+$ T cells are able to mediate antibacterial immunity in the absence of a concomitant granulomatous inflammation in infected tissues. The rapidly expressed T cell-mediated protection that can be observed under these conditions was, therefore, argued to be the result of a rapid, cytokine-induced activation of resident macrophages (29). To test this assumption, we used the monoclonal antibody (MAb) 5C6 (9, 46, 48–50), which has been shown to inhibit the recruitment of myelomonocytic cells into inflammatory foci and to exacerbate a primary listeria infection by binding to complement receptor type 3 (CR3, Mac-1, CD11b). If, in fact, listeria-primed CD8$^+$ or double-negative T cells act independently of invading phagocytes by rapidly activating resident macrophages for efficient listericidal activity, this antibody would have minimal effects on the outcome of a secondary infection in actively immunized mice. The present study, however, demonstrates that the inhibition of CR3-dependent migration of phagocytes dramatically increased the susceptibility to *Listeria monocytogenes* not only in a primary but also in a secondary infection, in which potent, granuloma-independent antibacterial mechanisms are expressed. However, two elements of T cell-mediated immunity were present even in the presence of 5C6 treatment and therefore seem to be CR3/CD11b independent: (i) protective immunity against low doses of viable listeriae and (ii) granuloma formation.

MATERIALS AND METHODS

Mice. Female mice of the inbred strain C57BL/6 were obtained from Jackson Laboratory, Bar Harbor, Maine, and kept under pathogen-free conditions in our own animal facilities. Mice were known to be free of common viral pathogens, as evidenced by the results of routine screening by the Institut fuer Versuchstierzucht, Hannover, Germany. Mice were used at the age of 8 to 12 weeks.

Bacteria and bacterial antigens. *L. monocytogenes* EGD was kept virulent by continuous mouse passages. Cultures were obtained by growing samples of spleen homogenates from infected mice in Trypticase soy broth, and aliquots of a log-phase culture were stored at −70°C until use. For each experiment, the appropriate number of vials from the *L. monocytogenes* stock suspension was thawed and diluted in phosphate-buffered saline (PBS) for intravenous (i.v.) inoculation. Heat-killed listeriae (HKL) were prepared by incubating *L. monocytogenes* in Trypticase soy broth for 18 h, washing the bacteria in PBS, and subsequently incubating the suspension containing 10^{10} CFU/ml for 1 h at 60°C.

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Sterility of each preparation was confirmed by incubating 0.1-ml aliquots in Trypticase soy broth overnight at 37°C and plating a sample of this culture on Trypticase soy agar.

MAbs with specificity to cell surface molecules. The MAb 5C6 (rat immunoglobulin G2b [IgG2b]) specific for CR3 of mouse myelomonocytic cells was generated as described previously (44). The antibody, purified by sodium sulfate precipitation, anion exchange, and exclusion chromatography, was injected i.v. in a dose of 800 mg per mouse. Three other rat MAbs (IgG2b), obtained from ascitic fluid from pristane-primed nude mice intraperitoneally (i.p.) injected with the relevant hybridoma line, were used. The hybridoma lines GK 1.5 (anti-L3T4), 2.43 (anti-Lyt-2), and 30-H12 (anti-Thy-1.2) were purchased from the American Type Culture Collection, Rockville, Md. (Tumor Immunology Bank 207, 210, and 107, respectively). MAb 23-7 (rat IgG, unrelated specificity) was kindly provided by T. Diamantstein. Ascitic fluid was cleared by centrifugation. The concentration of rat IgG2b was determined by radial immunodiffusion. Dilutions containing 500 μg/ml in PBS were filter sterilized (0.45-μm pore size) and stored at −70°C until use. Unless otherwise stated, MAbs were injected i.p. at a dose of 500 μg per animal on day −3 of the challenge infection. The effectiveness of T-cell subset depletion in vivo has been proved several times in our laboratory (34).

Antibodies with specificity for TNF-α and IFN-γ. The hamster anti-murine gamma interferon (IFN-γ) MAb used in this study was shown previously to inhibit macrophage activation in mice infected with *L. monocytogenes* (6) (P. code 1222-00; Genzyme, Boston, Mass.). It was injected i.p. at a dose of 150 μg per mouse (neutralizing capacity, ca. 5×10^4 U) in 0.5 ml of PBS containing 0.5% bovine serum albumin (BSA). The polyclonal rabbit anti-mouse tumor necrosis factor alpha (TNF-α) (P. code IP-400; Genzyme) was diluted 1:5 in PBS containing 0.5% BSA as the carrier protein before it was injected i.p. at a dose of 1 ml per mouse (neutralizing capacity of approximately 2×10^5 U).

Induction and challenge of immunity. Immunity to *L. monocytogenes* was induced by a primary infection with 10^4 bacteria injected i.v. in 0.2 ml of PBS. Immune mice were intravenously challenged with 5×10^5 to 1×10^6 viable bacteria 28 days after immunization. Viable bacteria were enumerated in the spleens of infected mice by plating 10-fold serial dilutions of organ homogenates on Trypticase soy agar and counting bacterial colonies after incubation for 24 h at 37°C.

Recall of granuloma formation in sensitized mice. Mice immunized by a primary infection 28 days previously (see above) were specifically challenged by an i.v. injection of HKL (2×10^8/200 μl). Three days later, challenged mice were sacrificed and livers were removed for histologic examination. Organs were formalin fixed, embedded in paraffin, sectioned (3 μm), and stained with hematoxylin and eosin by standard procedures.

Listeria-specific T-cell lines and clones. T-cell lines and clones were established as usual. Briefly, T-cell lines specific for *L. monocytogenes* or HKL were established from spleens or draining lymph nodes, respectively, obtained from C57BL/6 mice immunized with 10^4 viable bacteria or 100 μg of HKL in an emulsion of PBS and complete Freund adjuvant, respectively. Spleen (1×10^8) and lymph node (3×10^7) cells were cultured in 5 ml of RPMI 1640 medium (Biochrom, Berlin, Germany) supplemented with 2 mM glutamine, penicillin (50 U/ml), streptomycin (50 μg/ml), 5×10^5 M 2-mercaptoethanol (E. Merck, AG, Darmstadt, Germany), 10% fetal calf serum (Biochrom) (further referred to as enriched medium), and 1×10^8 HKL per ml. After 4 days of incubation, cells were washed and resuspended in 5 ml of the enriched medium described above without antigen but supplemented with 10% concanavalin A (ConA) (Sigma Chemical Co., St. Louis, Mo.) supernatant as a source of interleukin 2 as described previously. ConA supernatant was prepared from splenocytes of BALB/c mice by incubating 5×10^6 cells per ml with 5 mg of ConA per ml for 24 h, removing the supernatant, and blocking the remaining ConA with Sephadex G-50 (Pharmacia LKB, Uppsala, Sweden). The ConA supernatant was filter sterilized and stored at −70°C. T cells were kept in the ConA supernatant-enriched medium at a concentration of 1×10^6/ml and restimulated every 14 days as described previously, using 2×10^7 HKL per ml as the antigen presented by irradiated syngeneic spleen cells (2,200 rads). T-cell lines were cloned by the limiting dilution technique. Cells were diluted in enriched medium and distributed in wells of flat-bottom microtiter plates (Nunc, Roskilde, Denmark) at a concentration of 0.2 cells per 200 ml. Resulting clones were maintained as described above for the lines. Phenotypes of lines and clones ($CD4^+$) were determined by fluorescence-activated cell sorter analysis.

T-cell proliferation assay. Cloned T cells were used in proliferation assays 10 days after antigenic stimulation. T cells (10^4 per well) were cocultured with 0.5×10^6 irradiated syngeneic spleen cells (2,200 rads) and HKL in the presence or absence of various concentrations of MAb 5C6 diluted in enriched medium (see above). Cultures were set up in 200 μl of enriched medium in flat-bottom microtiter plates. At the end of a 48-h incubation, 0.5 mCi of [^{3}H]thymidine (5 Ci/mmol; Amersham, Braunschweig, Germany) was added. Eighteen hours later, cells were harvested and radioactivity was determined in a β-counter (Packard). Results are expressed as mean counts per minute of triplicates. Standard deviation values did not exceed 10% of the mean.

Adoptive transfer of immunity by listeria-specific T cells. In adoptive transfer experiments, mice infected with 3×10^4 *L. monocytogenes* cells 2 h previously were injected i.v. with 5×10^6 cells of a spleen-derived listeria-specific T-cell line. T cells were obtained from culture 4 days after an antigenic boost (see above), separated by Ficoll-Hypaque (Biochrom; specific density of 1.077 g/ml), washed, and suspended in sterile PBS at a concentration of 2.5×10^7/ml. Three days after infection, the bacterial load per spleen was determined (see above).

RESULTS

MAb 5C6 (anti-CD11b/CR3) prevents the expression of acquired immunity in actively immunized mice challenged with a high dose of *L. monocytogenes*. Mice of the listeria-resistant strain C57BL/6 were immunized i.v. with 10^4 viable listeriae per animal. Four weeks later, groups of five mice each were treated with MAbs specific to L3T4, Lyt-2, and Thy-1 (all rat IgG2b), respectively, or an irrelevant control rat MAb injected i.p. (control 1) or i.v. (control 2). Three days later, these and an additional group of mice injected i.v. 1 h previously with MAb 5C6 were challenged by an i.v. injection of 5×10^5 viable listeriae. Nonimmunized animals injected with the aforementioned challenge dose of bacteria or a low dose of listeriae (5×10^3 per animal) in the presence or absence of MAb 5C6 served as controls. Three days later, the bacterial load per spleen was determined. As shown in Fig. 1, all 5C6-treated, secondarily infected mice died, whereas potent antibacterial mechanisms were still ex-

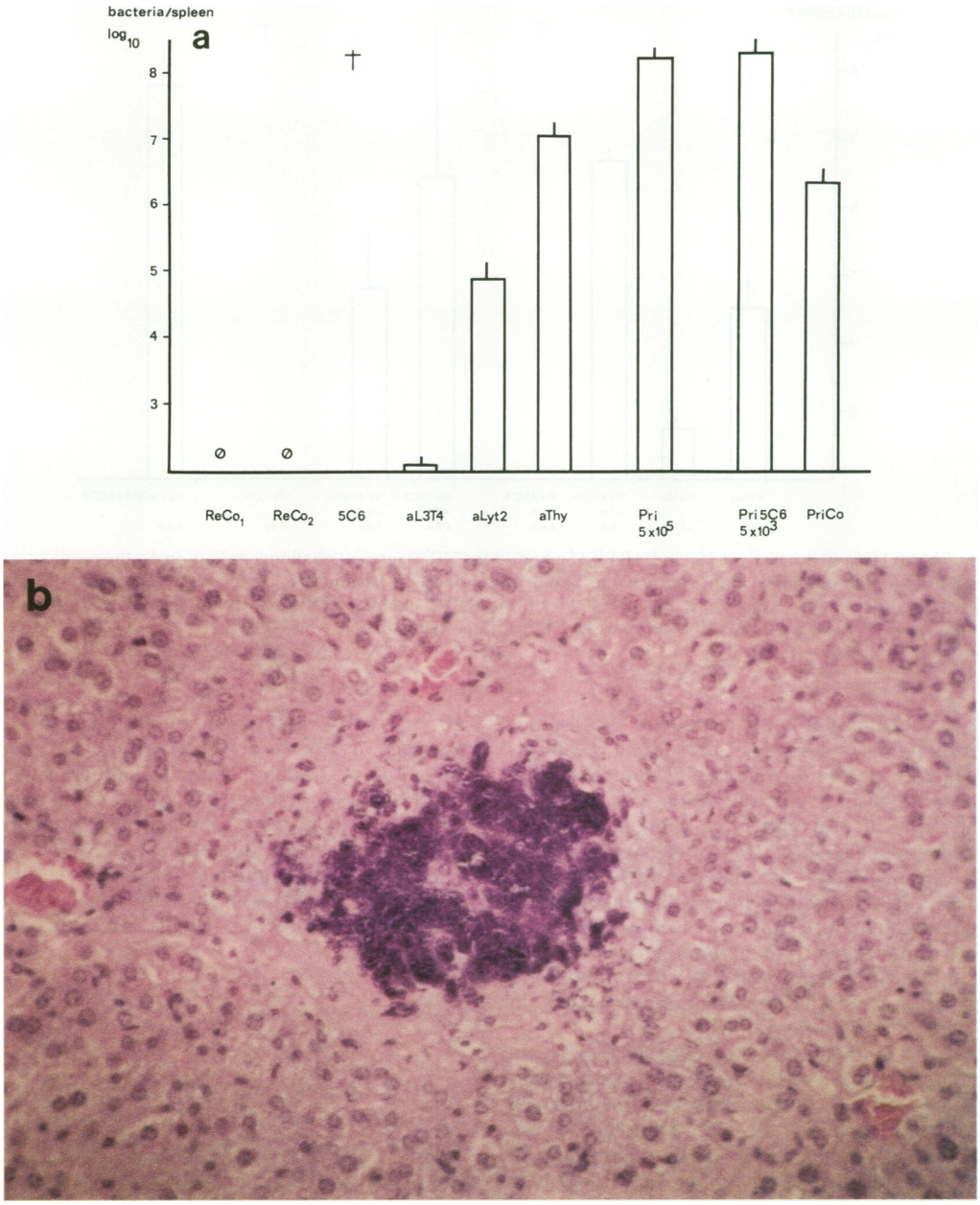

FIG. 1. (a) Numbers of bacteria in spleens (day 3) of secondarily infected mice (5×10^5 *L. monocytogenes* cells per animal) pretreated with MAbs specific to CR3 (5C6), L3T4, Lyt-2, or Thy-1.2. Animals treated i.p. ($ReCo_1$) or i.v. ($ReCo_2$) with an irrelevant rat MAb as well as primarily infected animals (Pri) infected with 5×10^5 or 5×10^3 bacteria in the presence (Pri5C6) or absence (PriCo) of MAb 5C6 served as controls. †, all mice died. ∅, $<10^2$ bacteria per organ. (b) Histomorphology of livers from secondarily infected but 5C6-treated animals 36 h after challenge with 5×10^5 viable bacteria. Infectious focus showing heavily infected hepatocytes.

pressed in $CD4^+$ T cell-depleted mice. Even primarily infected or totally T cell-depleted mice were still alive when 5C6-treated animals had already succumbed to infection. The histologic appearance of the livers of 5C6-treated immunized mice resembled that seen in primarily infected animals (48) (Fig. 1b) with the inhibition of both myelomonocytic cell and lymphocyte migration to foci of infection.

CR3-dependent mechanisms of immunity are expressed early after reinfection. To determine the kinetics of CR3-dependent mechanisms in the expression of acquired immunity, we challenged actively immunized mice with 10^6 listeriae per animal in the absence or presence of MAb 5C6 injected i.v. at −1, +16, or +24 h of the secondary infection. Figure 2 shows that as soon as 16 h after challenge, the bacterial load per spleen was significantly higher in mice treated 1 h before challenge, and all pretreated mice died within 60 h. The susceptibility to 5C6 treatment rapidly waned during the first 24 h of a secondary infection as demonstrated by the bacterial numbers in the spleens of mice treated 16 or 24 h after challenge.

MAb 5C6 does not inhibit T cell–antigen-presenting cell interactions in vitro. It has been shown (12, 52) that MAbs

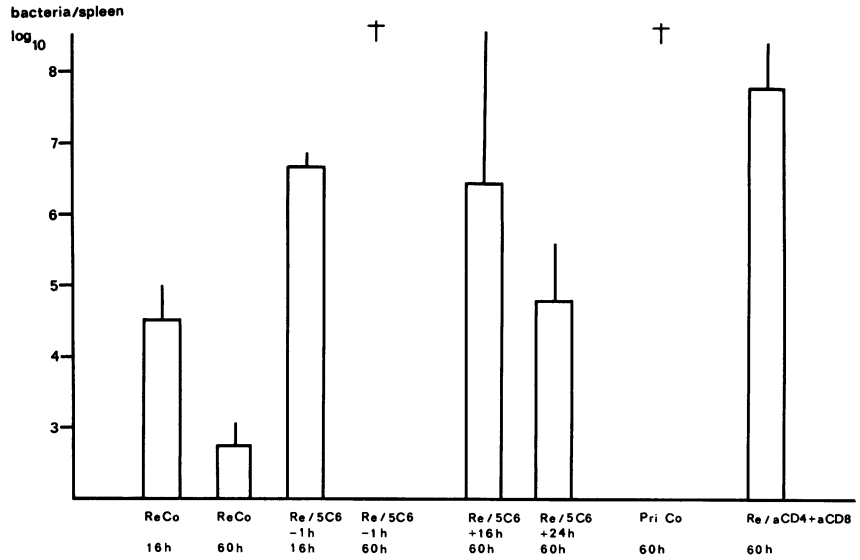

FIG. 2. Effect of MAb 5C6 given at −1, +16, and +24 h of a high-dose (10^6 bacteria) challenge infection on the eradication of bacteria from spleens of secondarily infected animals. Numbers of bacteria per spleen were determined at 16 and 60 h postinfection. The results obtained in secondarily infected control mice (ReCo) and mice depleted of both $CD4^+$ and $CD8^+$ T cells (Re/aCD4+aCD8) as well as those obtained in primarily infected mice (PriCo) are shown for comparison. †, all mice died.

with specificity to CD18 inhibit antigen-induced T-cell functions by interfering with T cell–antigen-presenting cell interaction. To investigate whether the effect of 5C6 might be due to the inhibition of memory T-cell activation, we tested for an effect of the antibody on antigen-stimulated, antigen-presenting cell-dependent proliferation of a listeria-specific T-cell clone. Cells of the $CD4^+$ listeria-specific T-cell clone THKL-C7 were cultured together with irradiated syngeneic spleen cells in the presence of HKL and various concentrations of MAb 5C6 or normal culture medium. However, over a wide range of antibody concentrations (0.1 to 50 μg/ml), no effect on antigen-stimulated [^{3}H]thymidine incorporation in vitro could be observed (data not shown).

Anti-CR3 MAb 5C6 inhibits expression of protective immunity against an intermediate dose of bacteria in mice adoptively immunized by a preactivated granuloma-inducing T-cell line. We determined the effect of the antibody on the expression of adoptively transferred immunity using cells (5×10^6 per animal) of an in vitro-stimulated listeria-specific T-cell line (ReKoHKL/spleen) known to transfer protection and granuloma formation. Even under these conditions, MAb 5C6, given 1 h before bacterial challenge (3 h before cell transfer), abolished the expression of $CD4^+$ T cell-mediated antilisterial mechanisms when mice were challenged with 3×10^4 viable listeriae (Fig. 3).

Acquired immunity prevents lethal outcome in 5C6-treated animals challenged with a low, usually immunizing dose of *L. monocytogenes*. It has been shown previously (48) that treatment with MAb 5C6 resulted in the death of nonimmune mice even when they were infected intradermally with as few as 10 viable listeriae. To assess the quantitative contribution of CR3-independent mechanisms in T cell-mediated protection, we compared the resistance of primarily and secondarily infected mice to various inocula of *L. monocytogenes*. Groups of immune and nonimmune mice were treated with MAb 5C6 1 h before challenge infection. The survival rate in each group is shown in Fig. 4. Whereas all primarily infected and 5C6-treated mice died after 48 h (5×10^5 or 5×10^4 bacteria per animal), 60 h (5×10^3 bacteria per animal), or 7 days (5×10^2 bacteria per animal), all immunized but 5C6-treated animals challenged with 5×10^3 and 5×10^2 listeriae survived. All 5C6-treated animals, regardless of whether they were immune or not, challenged with 5×10^4 bacteria died within 60 h.

HKL-induced granuloma formation is prevented by anti-TNF-α or anti-IFN-γ antibody treatment but not by treatment with anti-CR3 MAb. To be able to demonstrate the histomor-

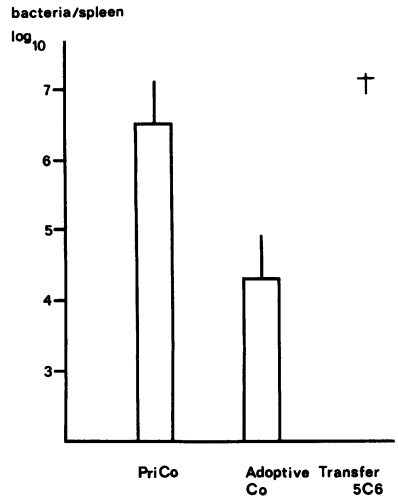

FIG. 3. Numbers of bacteria per spleen in normal, primarily infected mice (PriCo) and mice adoptively protected by the transfer of 5×10^6 cells (2 h after bacterial challenge with 3×10^4 bacteria) of a listeria-specific $CD4^+$ T-cell line specifically prestimulated in vitro. Results were obtained by enumerating viable bacteria in the spleens (day 3) of T-cell recipients in the presence (5C6) or absence (Co) of antibodies with specificity to CR3 injected i.v. 1 h before bacterial challenge. †, all mice died.

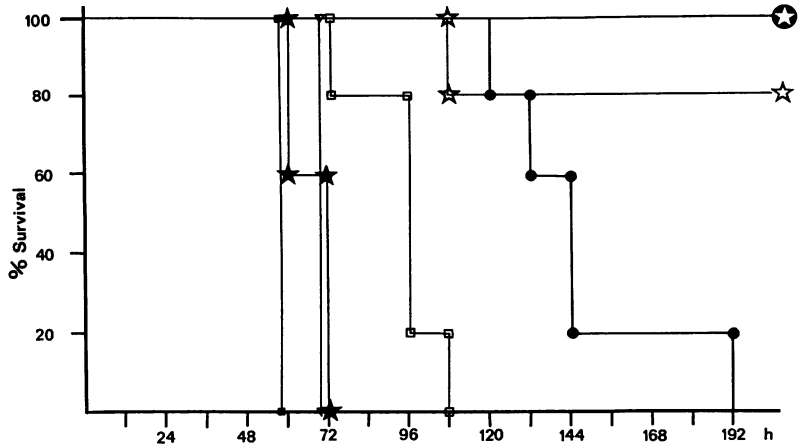

FIG. 4. Survival of primarily or secondarily listeria-infected mice in the presence or absence of anti-CR3 MAb 5C6. Primary infection control: 5×10^3 bacteria (☆) and 5×10^4 bacteria (□) per mouse. Primary infection plus MAb 5C6: 5×10^2 bacteria (●), 5×10^3 bacteria (★), and 5×10^4 bacteria (■) per mouse. Secondary infection control: 5×10^5 bacteria per mouse (✪). Secondary infection plus MAb 5C6: 5×10^3 bacteria (✪) and 5×10^4 bacteria (▽) per mouse. Whereas all 5C6-treated mice primarily infected with 5×10^3 bacteria died within 72 h (★), all immunized and 5C6-treated mice challenged with the same dose of bacteria survived (✪).

phology of the secondary immune response in mice treated with MAb 5C6 but protected against a low secondary challenge dose of *L. monocytogenes*, we used HKL for challenge (4). A total of 2×10^8 HKL (according to the bacterial load in primarily infected animals) were injected i.v. into mice immunized 4 weeks previously and treated with anti-CR3 1 h before challenge. At 36 and 60 h later, mice were sacrificed and livers were removed for histologic investigation. Both immune control and 5C6-treated mice showed a marked granulomatous reaction in response to HKL (Fig. 5). On the contrary, the ability to form granulomas was markedly reduced in mice pretreated with anti-TNF-α (neutralizing capacity, 2×10^5 U) or anti-IFN-γ (150 μg per animal) 2 h before challenge (Fig. 6). In control mice immunized 4 weeks earlier but sacrificed for histologic investigation without having been challenged, as well as in nonimmune mice challenged with HKL, no granulomas could be demonstrated.

DISCUSSION

This study substantiates and extends our previous observation (33–35) made in T-cell subset-depleted mice that granuloma formation and the most potent protective mechanisms expressed early after secondary listeria infection are mediated by distinct types of T cell-phagocyte interactions. In particular, the mechanisms underlying T cell-mediated protection in the absence of $CD4^+$ T cells as well as an apparent granulomatous inflammation were investigated to test the hypothesis that immunity could result from a rapid interaction of the remaining subsets with resident macrophages activating them for listericidal capacity. The results of this study, rejecting this hypothesis, can be summarized as follows.

(i) Listeria-primed $CD8^+$ T lymphocytes, possibly in conjunction with double-negative T cells, rapidly mediate protection by mechanisms dependent on an early interaction with myelomonocytic cells invading infected tissues within the first 24 h after reinfection in a CR3/CD11b-dependent manner (Fig. 1 and 2). This T cell-phagocyte interaction does not result in granuloma formation, although these cells have been shown to secrete TNF-α and IFN-γ in response to listerial antigen (36).

(ii) Listeria-specific $CD4^+$ T cells act mainly via a less protective but longlasting interaction with monocytes, resulting in delayed-type hypersensitivity and granuloma formation which is CR3/CD11b independent (Fig. 3 to 5) but nevertheless TNF-α and IFN-γ mediated (Fig. 6). The ability of $CD4^+$ T cells to induce granulomas is obviously independent of a preceding nonspecific myelomonocytic cell infiltration, which is prevented by anti-CR3 (5C6) MAb treatment (46).

(iii) The early CR3/CD11b-mediated influx of myelomonocytic cells into infected tissues contributes to resistance even in the absence of $Thy\text{-}1^+$ cells in anti-Thy-1.2 MAb-treated animals (Fig. 1).

In the murine listeriosis model (31, 37), three phases of phagocyte contribution to the resolution of infection have been described. Early phenomenological studies (42–44) of the primary infection have shown that *L. monocytogenes* is rapidly eliminated from the bloodstream, mainly by resident macrophages of the liver which have the potential to kill most of the bacteria without need for an immunological stimulus. However, approximately 20% of ingested bacteria escape from the phagosomes of fixed phagocytes and finally cross the sinusoid wall to infect neighboring parenchymal cells in which they multiply exponentially for 2 to 3 days because of their ability to secrete listeriolysin (9, 38, 48, 54). During this period, infective foci become heavily infiltrated with neutrophils which, despite their large numbers, cannot prevent bacterial growth. However, as demonstrated most clearly by North and colleagues (9, 13) and Bancroft and coworkers (2, 3), neutrophils and natural killer cells are active in restricting bacterial proliferation before listeria-sensitized T cells mediate an increase in number and bactericidal activity of macrophages which is thought to be ultimately responsible for the elimination of listeriae from the host. It was concluded mainly from autoradiography and irradiation experiments (43, 44, 53, 55, 56) that monocytic cells from the blood are the precursors of the majority of accumulating macrophages which, in collaboration with neutrophils, express resistance against listeriae.

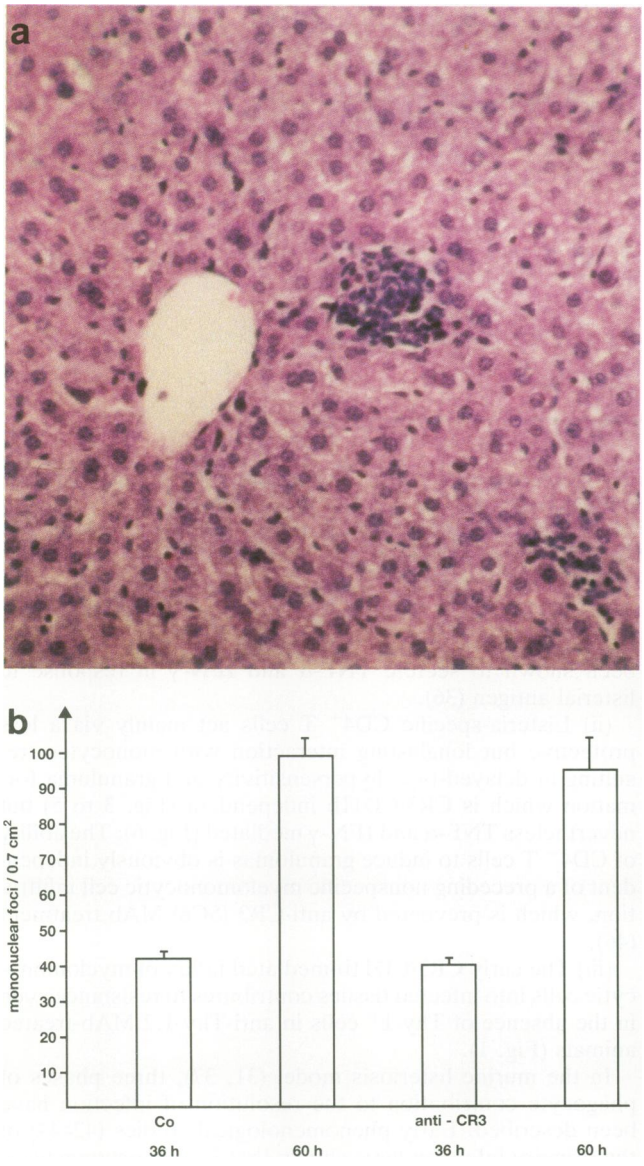

FIG. 5. (a) Histomorphology of livers from immune mice pretreated with MAb 5C6 and subsequently injected i.v. with HKL (2×10^8 per animal). Typical intraparenchymal focal accumulation of mononuclear cells. (b) Numbers of mononuclear foci (as demonstrated in panel a) in sections from five corresponding liver lobules each from control (Co) and anti-CR3 (5C6) MAb-treated mice. Livers were removed from immune mice challenged with HKL 36 and 60 h previously.

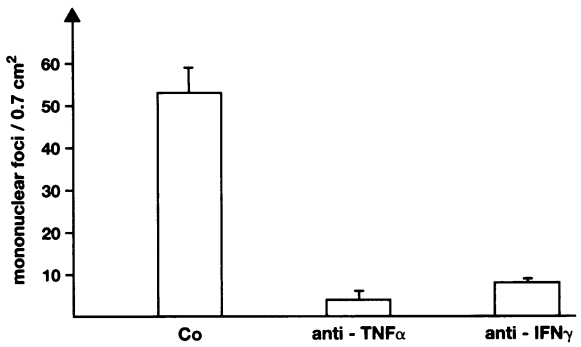

FIG. 6. Numbers of mononuclear foci in sections from five corresponding liver lobules each from immune, HKL-challenged (2×10^8 per animal) control (Co) and anti-TNF-α or anti-IFN-γ MAb-treated mice. Livers were removed 48 h after HKL challenge.

The egress of phagocytic cells from the bloodstream to infected tissues is known to require both chemotactic attraction (19) and intimate contact between leukocytes and vascular endothelium which is mediated mainly by members of the leukocyte integrin family of adhesion molecules (1, 7). Consequently, the MAb 5C6, specific for CR3/CD11b and able to inhibit myelomonocytic cell extravasation, totally eliminated the host's capacity to control bacterial multiplication in primary listeria infection (9, 46, 48, 49). Genetic studies (16, 17) revealed that the mechanisms determining the degree of innate resistance are closely linked to gene loci controlling the level of the C5 component of complement. Indeed, the low myelomonocytic inflammatory response in susceptible mice could be increased by the infusion of C5-rich serum.

The studies discussed so far investigated the contribution of phagocytes to resistance during the early phase of a primary infection and therefore explored their role in the expression of innate resistance rather than in acquired immunity able to protect mice against more than 100-fold-higher numbers of bacteria. Jungi and McGregor (25) were the first to examine the influx of monocytes in listeria-immune animals and presented evidence that monocyte accumulation at delayed inflammatory sites can occur under circumstances under which chemotactic activity cannot be detected in serum or in situ. In keeping with this observation, C5a-deficient, listeria-susceptible A/J mice were shown to be able to recruit as many inflammatory macrophages as C57BL/6 mice when immunized mice were investigated (10). The observed dissociation of local monocyte chemotactic activity and T cell-induced macrophage accumulation raised the question whether sensitized lymphocytes promote the focal deployment of monocyte-derived macrophages principally by mechanisms other than chemotaxis, e.g., by promoting their retention at sites of antigen injection after constitutive, random extravasation. In this context, it is worth mentioning that migration of monocytes from the blood into normal tissue sites is CR3 independent (47), as is listeria-induced granuloma formation (Fig. 5). In conclusion, while C5a seems to be the major chemotactic signal in the very early phase of listeria-induced inflammation, resulting in CR3-mediated phagocyte tissue invasion and resistance, the $CD4^+$ T cell-derived mediator of the CR3-independent accumulation of monocytes in granulomatous foci is unknown.

Previous studies in the listeriosis model (6, 13, 18, 20, 21, 41, 51) have shown that treatment with antibodies neutralizing the biological activity of TNF-α or IFN-γ dramatically reduces the animal's capacity to overcome the infection. More recently, Kindler et al. (28) explored for TNF-α production within granulomas formed in *Mycobacterium bovis* BCG-infected mice of a genetically susceptible strain and determined the effect on the evolution of these lesions after injecting an anti-TNF-α antibody. A major local source of TNF-α was found to be the activated macrophages within the granulomas. Antibody treatment not only impaired the induction of granulomas but also prevented the synthesis of TNF-α as well as the persistence of mature granulomas and

allowed a massive, sometimes lethal, proliferation of the infecting bacteria. These investigators concluded that macrophage TNF-α might play an autocrine and autoamplifying role in the generation of granulomas and their bactericidal properties. However, a T cell-derived mediator initiating the monocyte accumulation was not defined. In this respect, IFN-γ has been demonstrated to play a major role in leukocyte recruitment into cutaneous delayed-type hypersensitivity reactions (8, 15, 24). The intradermal injection of IFN-γ was active in recruiting leukocytes into the skin, and a MAb to IFN-γ inhibited delayed-type hypersensitivity reactions induced by the injection of supernatants obtained from mitogen-stimulated T cells or by the injection of specific antigen.

In view of the obvious role of TNF-α and IFN-γ in granuloma formation, demonstrated by us (this study) and others (28), it remains difficult to understand why $CD8^+$ cells, able to secrete both cytokines (5, 36) and to induce major histocompatibility complex class II expression in vivo (33 and unpublished data), do not induce granulomas in listeria-infected as well as in brucella-infected animals (33). In murine listeriosis, kinetic differences in the activation of $CD4^+$ and $CD8^+$ T cells could account for this observation. As recently shown (5), stimulation of $CD8^+$ T cells depends on viable, listeriolysin-producing bacteria, whereas $CD4^+$ T cells can be activated by both viable and killed bacteria, which could explain a prolonged stimulation of the latter T-cell subset, probably important for granuloma formation. This, in fact, is substantiated by different kinetics of the increase of numbers of $CD4^+$ and $CD8^+$ T cells in granulomatous lesions (40 and unpublished data). However, as shown in the brucellosis model (33), $CD4^+$ T-cell depletion abolished granuloma formation even in the presence of viable bacteria, and nude as well as SCID mice infected with listeriae show marked and longlasting production of TNF-α and IFN-γ in the presence of viable bacteria but nevertheless do not form granulomas (2, 3, 11, 18).

Taken together, the data presented here point to the presence of an as yet undefined $CD4^+$ T cell-derived factor initiating granuloma formation by the induction of CR3/CD11b-independent pathways of monocyte recruitment. The identification of the respective mechanisms would offer the chance to therapeutically manipulate T cell-induced monocytic inflammations without abolishing phagocyte-dependent mechanisms of resistance. The fact that $CD8^+$ T lymphocytes, alone or in collaboration with double-negative T cells, are able to mediate protection in the absence of granulomas but nevertheless depend on an interaction with myelomonocytic cells transitorily invading infected tissues in a CR3/CD11b-dependent manner, favors the hypothesis (26, 27) that these cells contribute to the exposure of intracellularly protected bacteria to the bactericidal mechanisms of activated blood-borne monocytes.

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