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Effect of Genetic Variation on Induced Neutrophilia in Mice

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Mice from a variety of strains were injected with a sterile irritant (Brewer's thioglycolate) and killed bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, or *Escherichia coli*) to determine their effect on accumulation of neutrophils in the peritoneal cavity. Peak accumulation occurred around 15 h postinjection and showed significant strain-related variation. C57BL/10 mice were identified as having a high-responder phenotype and BALB/c mice a low-responder phenotype. Inheritance of the high-responder phenotype followed simple Mendelian genetics: (BALB/c × C57BL/10)F_1 mice were found to be more responsive than either parental phenotype. Major histocompatibility complex *H-2*^{d} haplotype was found to convey an augmented neutrophil response in conjunction with B10 background high-responder genes (B10.D2/n) but the *H-2*^{d} haplotype per se was not the only factor in determining high responsiveness. Gram-positive and gram-negative bacteria appeared to activate different immune mechanisms. Both gram-negative bacteria and lipopolysaccharides (LPS) induced a response similar to, but less potent than, that induced by Brewer's thioglycollate. Neutralization of the LPS content of Brewer's thioglycolate abrogated the response.

Neutrophils have important effector cell activity against bacterial, fungal, and parasitic infections and are closely involved in the generation of inflammatory responses. Whilst neutropenia is associated with an increased risk of infection, overproduction or dysfunction of neutrophils can result in a number of important pathological conditions such as adult respiratory distress syndrome, cystic fibrosis (9), emphysema, or late-phase asthma.

Neutrophils are produced in the bone marrow and released into the peripheral blood, from where they pass into the tissues by extravasation to accumulate at sites of inflammation. Evidence exists to suggest that the production, mobilization, accumulation, and function of neutrophils vary in normal individuals as well as in individuals with specific genetic defects (e.g., Chediak-Higashi syndrome in humans [11] and *beige* mutation in mice) or acquired pathological abnormalities (7). The normal range of resting neutrophils in humans is strikingly broad, and some ethnic groups (Africans, Afro-Caribbeans, Yemenite Jews) demonstrate significantly lower levels than normal (19).

Although data showing genetically determined variation in neutrophil numbers exist for rats (22) and cattle (12), most experimental evidence is available for mice. Strain-dependent variation in the ability to mount neutrophil responses to a range of stimuli has been documented for a variety of mouse strains (1, 5, 6, 8, 20, 21). Similarly, strain-related differences in the accumulation of neutrophils in the peritoneal cavity have been observed following injection of sterile irritants (8, 20). Differences have also been reported in response to infection with organisms such as bacteria, fungi, and worms. For example, C57BL/6 mice mounted a threefold greater neutrophil response to killed *Listeria monocytogenes* than did A/J mice (5, 6). The extent of neutrophil infiltration into lung lesions correlated with high or low levels of responsiveness to infection with *Paracoccidioides brasiliensis* (3), and marked strain differences have been shown in neutrophil responses to the nematodes *Heligmosomoides polygyrus* and *Necator americanus* (1, 27).

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The causes of variation both in neutrophil responses to stimuli and in resting levels are presently unknown, but it is likely that genetically determined influences could act at all levels of production, mobilization, accumulation, and function. These influences may affect precursor or end cell populations, accessory cell function, or levels of factors controlling neutrophil production and function.

Here, we describe a murine model of genetic control of neutrophil responses. We have defined low- and high-responder mouse strains by challenge with a sterile irritant. The genetic inheritance of the high-response phenotype and its relationship to major histocompatibility complex (MHC) have been described. The pattern of neutrophil response to bacterial infections and to detoxified Brewer's thioglycolate have implicated bacterial endotoxin (lipopolysaccharide [LPS]) as the agent initiating this phenomenon. This model is currently being used to investigate the mechanisms underlying the observed variation, particularly with respect to the association between neutrophil and macrophage responses and the regulatory role of macrophages.

MATERIALS AND METHODS

Mice. Four- to five-week-old specific-pathogen-free female BALB.B, BALB.K, BALB/c, SWR, A/J, C57BL/10, B10.D2/n, and B10.BR mice were obtained from Harlan-Olac (Bicester, Oxon, United Kingdom). The strains were selected on the basis of their known variation in response to various stimuli (4, 8, 14, 20, 21, 28). For convenience, mice were used at 5 to 6 weeks old. Our own data (unpublished) show that responses at this age are similar to those in older mice. (BALB/c × C57BL/10)F_1 hybrid mice were bred in house from parents supplied by Harlan-Olac.

Induction of a leukocyte inflammatory response by injection of a sterile irritant. A local inflammatory response was induced by intraperitoneal (i.p.) injection of 1 ml of sterile 3% Brewer's thioglycolate (Difco). Mice were killed at defined intervals postinjection (p.i.) by chloroform inhalation. Peritoneal cells were harvested immediately by forceful injection of 4 ml of RPMI, gentle abdominal massage for 30 s, and withdrawal of 3.5 ml of fluid. To minimize effects of diurnal

variation, all samples were taken between 0900 and 1100 h. Viable cells were counted by using an improved Neuebauer chamber. Viability was assessed by the ability to metabolize fluorescein diacetate (10-ng/ ml), shown by fluorescence under UV light. Cells were centrifuged onto clean slides for 5 min, fixed in methanol, and stained with Wright's stain for 30 min, (1/4 in Sorensen's buffer). Differential cell counts were performed on at least 200 cells. As sham-inoculated (1 ml of pyrogen-free saline i.p.) animals were not statistically different from untreated animals at 18 h p.i. (data not shown), untreated animals were used as controls.

Induction of a leukocyte inflammatory response with biological agents. *Staphylococcus aureus* and *Staphylococcus epidermidis* were obtained from R. G. Finch, Department of Microbiology, City Hospital, Nottingham, United Kingdom. *Escherichia coli* (NM522) cultures were obtained from P. Tighe, University of Nottingham. The bacteria were plated for counting and then inactivated by overnight exposure to 4% formaldehyde in phosphate-buffered saline, washed three times in sterile 0.9% pyrogen-free saline, and stored at −80°C until required. Mice were injected i.p. with 1-ml volumes of varying concentrations of bacteria ranging from 10^4 to 10^{10} CFU/ml. Cytospins and viable cell counts were performed on peritoneal lavages at 18 h p.i. or at 1, 6, 18, and 24 h p.i. in the mice injected with 10^8 CFU of *S. aureus*.

Induction of a leukocyte inflammatory response by injection of bacterially derived LPS. A local inflammatory response was induced by i.p. injection of 1-ml volumes of LPS (*E. coli* serotype O111:B4; Sigma Chemicals, United Kingdom) in sterile saline, at concentrations up to 2,500 μg/ml. Peritoneal lavages were taken 15 h p.i. and examined as previously described.

Detection and neutralization of LPS in Brewer's thioglycolate. The LPS content of Brewer's thioglycolate was determined by the coagulation of *Limulus* amoebocyte lysate (E-Toxate; Sigma) and inactivated by incubation with polymyxin B-loaded agar beads (Sigma). The beads were first washed two times (2,000 rpm for 20 min) to remove preservatives, and then the pelleted beads from 10 or 100 μl of starting suspension (adsorptive capacity, 300 to 500 μg of LPS per ml of bead suspension) were added per ml of Brewer's thioglycolate. The beads were incubated overnight at 4°C before being removed by centrifugation. One milliliter of detoxified Brewer's thioglycolate was injected i.p., and peritoneal lavage was performed at 18 h p.i., as previously described.

Statistical analysis. The results were analyzed by the Mann-Whitney U test, and P values of less than 0.05 were considered significant.

RESULTS

Peritoneal neutrophil responses in mouse strains. The inflammatory response induced in the peritoneal cavity by Brewer's thioglycolate was maximal from 9 to 24 h p.i. in BALB/c mice and from 12 to 15 h p.i. in C57BL/10 mice (Fig. 1). The maximum neutrophil peak in C57BL/10 mice varied from 15 to 18 h p.i. in replicate experiments. Figure 2 shows the effects of injected Brewer's thioglycolate on the numbers of peritoneal neutrophils 18 h p.i. in four inbred strains. C57BL/10 mice were defined as high responders on the basis of their consistently high neutrophil count poststimulation. BALB/c and SWR mice showed an intermediate response, while A/J mice had a minimal response. BALB/c mice were selected as the low-responder strain for investigation because of the ready availability of congenic lines to facilitate genetic studies.

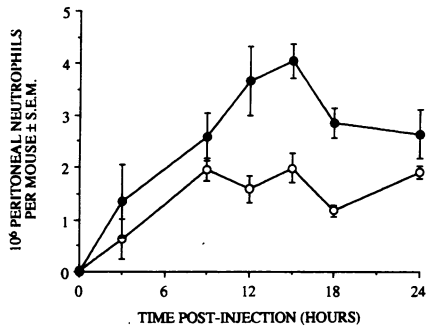

FIG. 1. Effect of time p.i. of Brewer's thioglycolate on peritoneal neutrophil response. BALB/c (○) and C57BL/10 (●) mice were injected i.p. with 1 ml of 3% Brewer's thioglycolate, and peritoneal lavages were performed at various times p.i. Results are shown as the number of peritoneal neutrophils per mouse, expressed as the mean for five mice ± SEM (bars). C57BL/10 mice are significantly more responsive than BALB/c mice (at 12 h, $P < 0.05$; 15 h, $P < 0.01$; and 18 h, $P < 0.01$).

Inheritance of response phenotype. Inheritance was examined in (BALB/c × C57BL/10)F_1 mice (Fig. 3A). The F_1 mice mounted a significantly higher peritoneal neutrophil response than either parent when inoculated with Brewer's thioglycolate.

Effect of MHC on neutrophil responses. The effect of MHC on neutrophil responses was examined in a number of congenic mouse lines carrying different MHC haplotypes on BALB or C57BL/10 background. On the BALB background, MHC haplotypes *H-2*^{b}, *H-2*^{d}, and *H-2*^{k} (BALB.B, BALB/c, and BALB.K, respectively) had no significant influence on peritoneal neutrophil response to Brewer's thioglycolate. However, on the C57BL/10 background, expression of the *H-2*^{d} haplotype (B10.D2/n) resulted in a significantly greater response, whereas expression of *H-2*^{b} (C57BL/10) or *H-2*^{k} (B10.BR) had no significant effect (Fig. 3B).

Expression of response phenotype during exposure to model infections. (i) Gram-positive bacteria. The maximal peritoneal responses elicited by *S. aureus* and *S. epidermidis* after 18 h were comparable. In BALB/c mice, the maximum neutrophil count induced by *Staphylococcus* spp. was similar to the response to Brewer's thioglycolate. The maximum response in C57BL/10 mice was comparable to that in BALB/c mice and

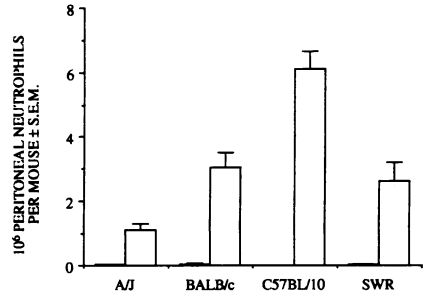

FIG. 2. Effect of Brewer's thioglycolate on peritoneal neutrophil response in four inbred mouse strains. One milliliter of 3% Brewer's thioglycolate (□) was injected i.p., and peritoneal lavages were performed 18 h p.i. Noninjected mice were used as controls (■). Results are shown as the mean number of peritoneal neutrophils per mouse ± SEM (bars) for three to six mice. All strains show significant responses compared with those of controls ($P < 0.05$).

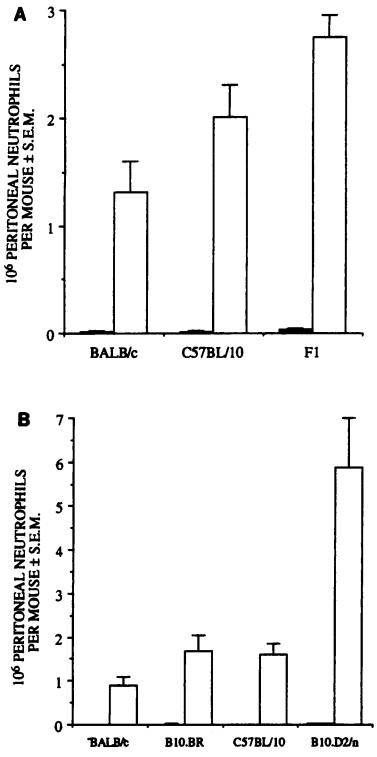

FIG. 3. Genetic influences on neutrophil response to Brewer's thioglycolate. (A) Inheritance of the response phenotype in (BALB/c × C57BL/10)F_1 hybrid mice. One milliliter of 3% Brewer's thioglycolate (□) was injected i.p., and peritoneal lavages were performed 18 h p.i. Noninjected mice were used as controls (■). Results are shown as the number of neutrophils per mouse expressed as the mean for 11 or 12 mice ± SEM (bars). These are the combined results of three experiments. F_1 mice are significantly better responders than either parent (F_1 versus BALB/c, $P < 0.01$; F_1 versus C57BL/10, $P < 0.01$). (B) Effect of MHC haplotype on neutrophil response to Brewer's thioglycolate in B10 congenic mouse strains. One milliliter of 3% Brewer's thioglycolate (□) was injected i.p., and peritoneal lavages were performed 18 h p.i. Noninjected mice were used as controls (■). Results are shown as the number of neutrophils per mouse, expressed as the mean for five or six mice ± SEM (bars). The *H-2*^{d} haplotype (B10.D2/n) conveys a significantly greater response than other congenic strains (*H-2*^{k} versus *H-2*^{d}, $P < 0.01$; *H-2*^{b} versus *H-2*^{d}, $P < 0.01$).

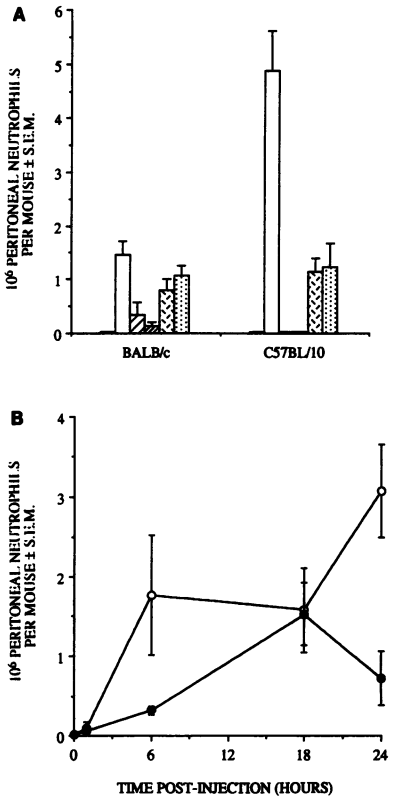

FIG. 4. Effect of killed *S. aureus* on peritoneal neutrophil response in BALB/c and C57BL/10 mice. (A) Effect of different concentrations of *S. aureus* on neutrophil response. One milliliter of killed bacteria, in a concentration of 10^4 (▨), 10^6 (▨), 10^8 (▨), or 10^9 (▨) CFU/ml, was injected i.p., and peritoneal lavages were performed 18 h p.i. The response to Brewer's thioglycolate (□) is included for comparison of the magnitudes of the responses. Noninjected mice were used as controls (■). Results are shown as the number of neutrophils expressed as the mean for five mice ± SEM (bars). At lower concentrations, BALB/c mice showed a greater response than C57BL/10 mice (10^4 CFU/ml, $P < 0.02$; 10^6 CFU/ml, $P < 0.01$). (B) Effect of time p.i. of *S. aureus* on neutrophil response. BALB/c (○) and C57BL/10 (●) mice were injected with 1 ml of *S. aureus* (10^8 CFU/ml), and peritoneal lavages were performed at various times p.i. Results are shown as the number of neutrophils per mouse, expressed as the mean for three or four mice ± SEM (bars). BALB/c mice showed the greatest responses at 6 ($P < 0.05$) and 24 ($P < 0.05$) h p.i.

significantly less than that attainable with Brewer's thioglycolate. BALB/c mice were more responsive to lower concentrations of *S. aureus* than were C57BL/10 mice (Fig. 4A). When the response to *S. aureus* at 10^8 CFU/ml was examined over a range of times from 1 to 24 h p.i., the peritoneal neutrophil response was significantly greater in BALB/c mice than in C57BL/10 mice at 6 and 24 h (Fig. 4B).

(ii) Gram-negative bacteria. BALB/c mice stimulated with killed *E. coli* showed a peritoneal neutrophil response comparable to that achieved with Brewer's thioglycolate (Fig. 5). C57BL/10 mice were significantly more responsive than BALB/c mice, although the peak response was less than that achieved with Brewer's thioglycolate.

Effect of LPS on neutrophil responses. There was a strain-related difference in the peritoneal neutrophil responses of C57BL/10 and BALB/c mice to concentrations of 500 to 2,500 μg of LPS (Fig. 6A). There was no discernable dose-dependent increase in response across this range with either strain, and the maximum yield for both strains was less than that achievable following stimulation with Brewer's thioglycolate.

Detection and inhibition of LPS in Brewer's thioglycolate. LPS was detectable in Brewer's thioglycolate at 10 to 20 ng/ml. Inactivation of the LPS content of Brewer's thioglycolate with polymyxin B resulted in a dose-dependent reduction of peritoneal neutrophil accumulation to levels which in C57BL/10 mice were close to, and in BALB/c mice were statistically the same as, those in unstimulated controls (Fig. 6B).

DISCUSSION

Following injection of a sterile irritant into the peritoneal cavities of mice, neutrophils accumulated over time. Peak accumulation occurred after about 15 h, in agreement with published results (6). Brewer's thioglycolate was the most potent of a range of commonly used inducers of inflammatory response. Mice from a number of inbred strains were injected,

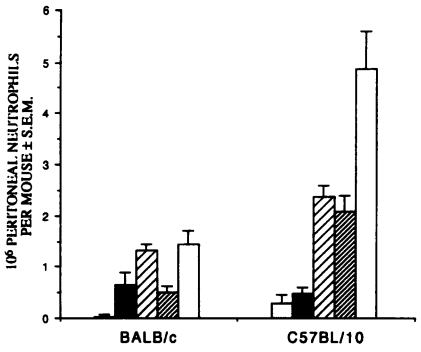

FIG. 5. Effect of killed *E. coli* on peritoneal neutrophil response in BALB/c and C57BL/10 mice. One milliliter of killed *E. coli*, in a concentration of 10^4 (▨), 10^6 (■), 10^8 (▨), or 10^{10} (▨) CFU/ml, was injected i.p., and peritoneal lavages were performed 18 h p.i. The response to Brewer's thioglycolate (□) is included for comparison of the magnitudes of the responses. Noninjected mice were used as controls. Control values were $(0.020 \pm 0.020) \times 10^6$ (BALB/c) and $(0.025 \pm 0.009) \times 10^6$ (C57BL/10) neutrophils per ml. Results are shown as the number of neutrophils per mouse, expressed as the mean for five mice ± SEM (bars). C57BL/10 mice are significantly more responsive than BALB/c mice at 10^8 ($P < 0.01$) and 10^{10} ($P < 0.01$) CFU/ml.

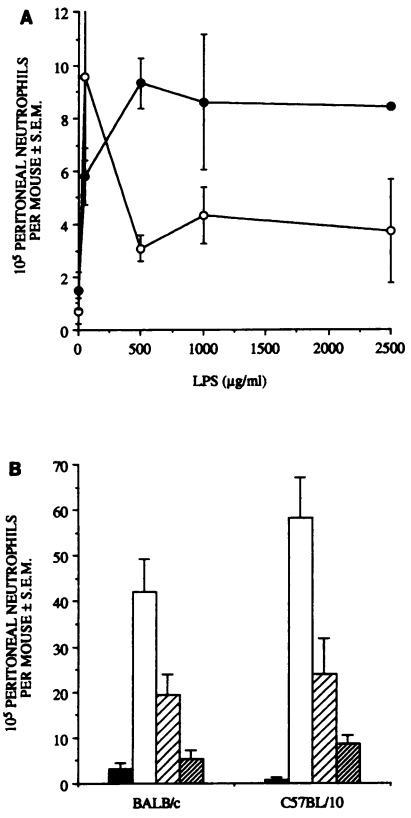

FIG. 6. Role of LPS in Brewer's thioglycolate-generated peritoneal neutrophil response. (A) Effect of LPS on peritoneal neutrophil response. BALB/c (○) and C57BL/10 (●) mice were injected i.p. with 1 ml of LPS at varying concentrations, and peritoneal lavages were performed 15 h p.i. Results are shown as the number of neutrophils per mouse, expressed as the mean for two to four mice ± SEM (bars). C57BL/10 mice show significantly greater neutrophil response to LPS at 500 μg/ml ($P < 0.02$). (B) Effect of polymyxin B-treated Brewer's thioglycollate on peritoneal neutrophil response. BALB/c and C57BL/10 mice were injected i.p. with either 1 ml of Brewer's thioglycolate (□), or thioglycolate that had been treated with 10 (▨) or 100 (▨) μl of polymyxin B per ml. Peritoneal lavages were performed 18 h p.i. Mice injected with 1 ml of pyrogen-free Hanks balanced salt solution were used as controls (■). Results are shown as the number of peritoneal neutrophils per mouse, expressed as the mean for five mice ± SEM (bars). Even 10 μl of polymyxin B per ml significantly reduced the neutrophil response elicited by Brewer's thioglycolate ($P < 0.02$).

and the peak peritoneal neutrophil responses showed significant strain-related variation. It is appreciated that study of four strains is insufficient to determine whether the response range is continuous or whether it can be truly categorized as low, intermediate, and high. However, for the purposes of experimental analysis these strains provided reproducible strain-related differences. C57BL/10 was chosen as the high-responder phenotype. Although A/J mice were significantly less responsive than BALB/c mice, the latter were selected as a low-responder phenotype because the ready availability of congenic BALB strains facilitated genetic studies and because the C5 deficiency known to exist in A/J mice could potentially affect neutrophil accumulation independently of other genetic effects. The pattern and magnitude of neutrophil response were not affected by mouse age up to at least 11 weeks (data not shown). Although there was considerable variation in the absolute numbers of peritoneal neutrophils generated between experiments, consistency of response rank order was maintained within experiments, with the response levels of C57BL/10 mice always 2.0-fold ± 0.2-fold higher than those of BALB/c mice. From a sample of 11 experiments using separate batches of mice, mean values ± standard errors of the mean (SEM) were $(2.1 \pm 0.4) \times 10^6$ (BALB/c) versus $(3.9 \pm 0.5) \times 10^6$ (C57BL/10). Matched-pair analysis of results (Wilcoxon signed rank) showed a highly significant difference ($P < 0.001$) between the two strains. Therefore, we conclude that while there are clear environmental effects on total neutrophil accumulation, they are consistent for the two strains and do not markedly influence the strain-dependent genetic variation observed. Time course studies showed that peritoneal neutrophil accumulation was first detectable at 2 h p.i. despite blood neutrophil levels (data not shown) being elevated in both strains within 30 min of injection. The differences in neutrophil accumulation between the C57BL/10 and BALB/c strains were apparent from 12 to 24 h p.i. It should be noted that response phenotype was not directly related to baseline neutrophil levels, with the high-responder C57BL/10 mice demonstrating significantly lower resting levels in the blood, marrow, and peritoneal cavity than other strains tested. Mobilization of neutrophils from the marginal pool was initially comparable in the two strains, with a peripheral blood neutrophilia within the first 9 h p.i. and a drop to the baseline level followed by a second increase at 12 h (data not shown). No significant changes in marrow neutrophil counts were recorded. Replicates of the time course experiment consistently showed that in stimulated BALB/c mice, peritoneal neutrophil numbers reached a plateau at 6 to 9 h p.i., while peritoneal neutrophil numbers in C57BL/10 mice continued to increase up until 15 to 18 h p.i.

We consider that the neutrophils that accumulate prior to 9 h p.i. are principally supplied by the marginal pool, as indicated by the rapid blood neutrophilia without attendant alteration in the total numbers of marrow neutrophils. The second peak in blood neutrophil numbers at 12 h may well indicate the onset of neutrophil production and release from

the marrow, particularly since serum colony-stimulating activity in C57BL/10 mice infected with live *E. coli* peaks at 9 h p.i. (10) while colony-stimulating activity production from macrophages stimulated in vitro is maximal from 6 to 12 h poststimulation (23). However, we found that the proportion of polymorphonucleocytes to myelocytes-metamyelocytes in the peritoneal cavity does not change across this period. Work is in progress to clarify this point.

The higher level of response of (C57BL/10 × BALB/c)F_1 mice than that of either parent strongly suggests complementation between genes present in each strain. In studies of congenic mouse lines expressing different MHC haplotypes, no differences in the neutrophil responses of BALB.B, BALB/c, and BALB.K (*H-2*^{b}, *H-2*^{d}, and *H-2*^{k} haplotypes, respectively) were detected. However, on the C57BL/10 background the *H-2*^{d} haplotype (B10.D2/n) showed an augmented neutrophil response which was significantly higher than those of the other congenics tested. Since BALB/c also expresses the *H-2*^{d} haplotype, it is clear that possession of this haplotype per se is not responsible for the very-high-response phenotype but that MHC-linked genes act in concert with background high-responder genes to enhance neutrophil response. Since the (C57BL/10 × BALB/c)F_1 hybrids were significantly more responsive than either parent, there was a possibility that the *H-2*^{d} haplotype inherited from the BALB parent was interacting with the B10 background. All of these data point to the conclusion that the neutrophil response phenotype is under polygenic control.

The neutrophil response patterns of the low- and high-responder mouse strains were compared, with bacteria as the biological stimulus. In mice infected with live *L. monocytogenes* (4, 14), peripheral neutrophilia and particularly levels of colony-stimulating factors in serum were higher in susceptible than in resistant animals, despite accumulation of neutrophils at the site of infection being greater in resistant animals. This was attributed to the reduced magnitude of immunogenic stimulation in the more competent host and paralleled our own observation with *Trypanosoma musculi* in this laboratory (unpublished observations), in which the resistant (C57BL/10) mice mounted a significantly smaller peritoneal inflammatory response than the susceptible (BALB/c) strain. To prevent such variation in stimuli, the bacteria were fixed in formalin before i.p. injection. Both gram-positive and gram-negative bacteria were examined, since they trigger different host responses. The two *Staphylococcus* species, *S. aureus* and *S. epidermidis*, proved to be equally effective at high concentrations in stimulating a peritoneal neutrophil response. However, the maximum response in the high-responder C57BL/10 mice after 18 h was no greater than that of the low-responder BALB/c mice despite the C57BL/10 strain having a demonstrably greater absolute capability to generate neutrophils. C57BL/10 mice may be less sensitive to stimulation by *Staphylococcus* spp. than are BALB/c mice, which produced significantly more peritoneal neutrophils in response to low levels (10^4 to 10^6 CFU/ml) of *S. aureus*. It is doubtful that increasing the inoculum size beyond 10^9 bacteria would result in further neutrophil accumulation in C57BL/10 mice. However, when the response to *S. aureus* at 10^8 CFU/ml was examined over 24 h, BALB/c mice showed significantly greater neutrophil responses than C57BL/10 mice at several time points. This suggests that the mechanisms by which Brewer's thioglycolate induces a superior neutrophil response in C57BL/10 mice are not activated by gram-positive bacteria.

With a gram-negative organism, *E. coli*, the peak response in C57BL/10 mice was significantly greater than that in BALB/c mice, although still less than that achievable with Brewer's thioglycolate. Responses in both strains apparently peaked at an inoculum size of 10^8 bacteria. It is therefore probable that *E. coli* activates at least part of the high-responder phenotype response mechanisms. A likely bacterially synthesized stimulus for this activation would be LPS. LPS was detectable in Brewer's thioglycolate at 10 to 20 ng/ml, sufficient to induce cytokine release from leukocytes in vitro (23). However, when the effects of a range of LPS concentrations were observed in mice 15 h p.i., >50 μg of LPS per ml was required to induce a maximal peritoneal response. Assaying the endotoxin standard with serial dilutions of Brewer's thioglycolate showed the Brewer's thioglycolate to contain inhibitors of the *Limulus* amoebocyte assay, and we conclude that the effective LPS content of the irritant is significantly greater than the measured value. C57BL/10 mice mounted a significantly higher-magnitude response than BALB/c mice to >500 μg of LPS per ml i.p., although the magnitude of response was lower than that achieved with Brewer's thioglycolate. In agreement with others (23), we found that increases in LPS concentration above the response threshold did not further increase the inflammatory response. At low concentrations (1 to 100 ng/ml) LPS produced a minimal decrease in peritoneal cellularity (data not shown).

Polymyxin B binds to bacterial LPS (16) and when chemically bonded to insoluble agar beads can be used to remove LPS from a solution. When the LPS content of Brewer's thioglycolate was removed by polymyxin B, there was a dose-dependent reduction in peritoneal neutrophil response to near baseline levels. Therefore, the inflammatory mechanism which we have described is LPS triggered. The presence of inhibitors makes it difficult to measure the concentration of LPS in Brewer's thioglycolate accurately. Thus, whereas sufficient polymyxin B to remove 3 to 5 μg of LPS per ml reduced peritoneal response by only approximately 50%, amounts sufficient to remove 30 to 50 μg/ml were almost 100% effective.

The data presented here describe a genetically determined variation in the ability of mice to generate peritoneal neutrophilia and characterize a reproducible experimental model in which the mechanisms underlying this variation can be analyzed. The generation of neutrophilia following stimulation of the peritoneal cavity involves a complex sequence of events, in which the products of a number of different cells may be active (24). The rapidity of the response implies that resident cells such as macrophages, endothelial cells, and mast cells (2) are likely to be the source of the cytokines and other mediators concerned, but variation may be expressed at the level of the bone marrow response to these factors, as has been described for eosinophils and mast cells (25). Macrophages in particular are known to mediate LPS-generated neutrophilia. LPS induces macrophages in vitro to release colony-stimulating factors (15, 23), interferon (26) and the proinflammatory cytokines tumor necrosis factor (13) and interleukin-1 (17). Macrophages also produce chemokines. Chemokines are a newly identified superfamily of proteins which mediate inflammation via chemotaxis and activation of specific subsets of leukocytes.

This paper has described a useful model of genetic control of neutrophil accumulation in mice. The data raise the question of the mechanisms underlying this variation. It is interesting that macrophage responsiveness in the strains of mice used here (data not shown) reveals a pattern strikingly similar to that described for neutrophils, as has been reported previously (20). A recent report, showing that the genetic control of macrophage responses is also polygenic (18), suggests that there may be functional correlations between the responses

seen in the two cell populations. This aspect is currently being investigated.

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