129X1/SvJ Mouse Strain Has a Novel Defect in Inflammatory Cell Recruitment

Peter White, Stephen A. Liebhaber and Nancy E. Cooke

*J Immunol* 2002; 168:869-874; 
doi: 10.4049/jimmunol.168.2.869

http://www.jimmunol.org/content/168/2/869

---

**References** This article cites 33 articles, 13 of which you can access for free at:
http://www.jimmunol.org/content/168/2/869.full#ref-list-1

**Subscription** Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions** Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
129X1/SvJ Mouse Strain Has a Novel Defect in Inflammatory Cell Recruitment

Peter White, Stephen A. Liebhaber, and Nancy E. Cooke

Vitamin D-binding protein (DBP) has been reported to contribute to innate immunity. To verify prior in vitro and cell-based observations supporting this role, we assessed the ability of a recently developed DBP-null mouse line to recruit neutrophils and macrophages to a site of chemical inflammation. The interrupted DBP allele had been generated by homologous recombination in 129X1/SvJ embryonic stem cells and these cells were subsequently used to generate a line of DBP−/− (null) mice. Initial studies revealed a marked defect in the ability of these DBP−/− mice to recruit cells to the peritoneum after localized thioglycolate injection. However, progressive outcrossing of the DBP−/− mice to the C57BL/6J strain, conducted to provide a uniform genetic background for comparison of DBP-null and control mice, resulted in a progressive increase in cell recruitment by the DBP−/− mice and a loss in their apparent recruitment defect when compared with the DPB wild-type controls. These data suggested that the observed recruitment phenotype initially attributed to the absence of DBP was not linked to the DBP locus, but instead reflected the underlying genetic composition of the 129X1/SvJ ES cells used for the initial DBP gene disruption. A profound cell recruitment defect was confirmed in the 129X1/SvJ mice by direct analysis. Each of three commonly used inbred lines was discovered to have a distinct level of cell recruitment to a uniform stimulus (C57BL/6J > BALB/c > CD1 > 129X1/SvJ). Thus, this study failed to support a unique role for DBP in cellular recruitment during a model inflammatory response. Instead, the data revealed a novel and profound defect of cell recruitment in 129X1/SvJ mice, the strain most commonly used for gene deletion studies. The Journal of Immunology, 2002, 168: 869–874.

The use of inbred or “isogenic” mouse strains has facilitated numerous advances in biology (1). Due to the generalized homozygosity in their genomes, physical differences among these mouse strains can be more directly related to underlying genetic determinants than would be the case for mice with mixed genetic constitution. However, unappreciated phenotypic differences between isogenic strains may complicate the interpretation of phenotypes resulting from the rapidly expanding array of targeted inactivated genes generated by homologous recombination (“knockout” mice; Refs. 2 and 3). Gene knockout studies inactivate specific loci in the mouse genome and set the stage for subsequent assessment of the impact of the specific gene mutation on phenotype. These studies are routinely conducted in embryo stem (ES) cells derived from the 129X1/SvJ mouse strain due to the highly efficient germline colonizing ability of its derived ES cells (4). In addition, the use of ES cells from an isogenic strain generally increases the efficiency of homologous recombination when matched with targeting vectors containing homology (targeting) arms derived from the same genome (5). Thus, knockout studies, although powerful in linking genes to function, have the potential to be confounded by the distinct genetic makeup of the isogenic strain, most frequently 129X1/Sv, from which the originating ES cells are derived.

Vitamin D-binding protein (DBP) is a highly expressed, polymorphic serum protein with functions ranging from the transport of vitamin D metabolites to roles in the immune system, host defense, and neutrophil chemotaxis (reviewed in Refs. 6–8). Recent studies from our laboratory have addressed central issues of DBP in the transport, metabolism, and function of vitamin D using a DBP-deficient (DBP−/−) mouse model (9). In addition to these well-documented vitamin D-related functions, a body of literature suggests that DBP also plays a pivotal role in innate immunity. In particular, DBP has been reported to augment chemotaxis of neutrophils, monocytes, and fibroblasts when studied ex vivo (10–12). This effect has been attributed to a C5a cochemotactic activity of DBP that requires binding of DBP to chondroitin sulfate proteoglycans on the cell surface of leukocytes (13–16). In light of these reports, we set out to test the role of DBP in recruitment of inflammatory cells in vivo using the DBP−/− mouse model.

Localized injection of thioglycollate medium (TG) into the peritoneum of test animals has been established as a straightforward and effective approach in examining inflammatory cell recruitment and for isolation of peritoneal macrophages (17, 18) and neutrophils (19). When TG is injected into the peritoneal cavity of mice or rats it induces localized sterile inflammation with subsequent recruitment of leukocytes in a characteristic, cell-specific pattern. Thermal “aging” of TG has been reported to increase its inflammatory effect due to the formation of advanced glycation end products that can accentuate cell recruitment (20). In contrast to other inflammatory agents such as LPS, TG can recruit large numbers of peritoneal leukocytes without activating microbicidal activity (21). The cell recruitment triggered by TG appears to operate via a complement-independent pathway, although the exact mechanism(s) of...
this established model of innate immune response remain poorly defined.
In the following study, we have used the TG experimental model to assay the putative role of DBP as a neutrophil or macrophage recruitment factor. The data argue against a unique role for DBP in TG-elicited peritoneal neutrophil or macrophage recruitment in vivo, and instead they reveal an unanticipated and profound defect on cell recruitment that reflects complex genetic determinants and is specific to the commonly used 129X1/SvJ iso-
genic mouse strain.

Materials and Methods

Mice

CD-1 mice were purchased from Charles River Breeding Laboratories (Wilmington, MA); all other strains where purchased from The Jackson Laboratory (JAX mice; Bar Harbor, ME). The animals were housed in Jag-75 plastic cages with wood shavings in a high efficiency particulate air-filtered barrier unit maintained at 25°C with alternating 12-h periods of light and dark. The mice had access to drinking water at all times and were fed ad libitum with an autoclaved pelleted rodent diet. The great majority of mice used were between 6 and 12 wk of age at the time of the experiment. Four strains of mice were used. 129X1/SvJ. The 129X1/SvJ inbred mouse strain originated from crosses established in 1928. It is the strain most commonly used to generate ES cells for gene knockout studies (22).

C57BL/6J. C57BL/6d is widely used, accounting for 14% of all occasions in which an inbred strain has been used in the literature (http://www.informatics.jax.org).

BALB/c. The BALB/c inbred mouse strain is commonly used in the field of immunology (23).

CD1. CD1 is a commonly used albino, outbred mouse strain originating from Switzerland.

Thioglycolate medium

Two variants of TG were tested: ready-to-use, aged TG from stored at 4°C (BD Bioscience, Franklin Lakes, NJ), and dehydrated Brewer TG medium (Difco, Detroit, MI). The latter was taken up as a 3% solution (30 g of TG powder in 1000 ml of deionized water) and was autoclaved 20 min at 15 lbs/psi (121°C). The sterile 3% TG was protected from light and was “aged” at room temperature for at least 1 mo to ensure formation of advanced glycation end products (20). Preprepared and laboratory-prepared TG were identical in their abilities to trigger peritoneal recruitment of macrophages in all four mouse strains studied (data not shown). All data presented in this report were obtained through the use of preprepared TG medium.

Peritoneal cell recruitment

For recruiting peritoneal cells, 1 ml of sterile TG medium (warmed to room temperature) was injected into the peritoneal cavity of male or female mice (Wilmington, MA); all other strains where purchased from The Jackson Laboratory (JAX mice; Bar Harbor, ME). The animals were housed in a light and dark. The mice had access to drinking water at all times and were fed ad libitum with an autoclaved pelleted rodent diet. The great majority of mice used were between 6 and 12 wk of age at the time of the experiment. Four strains of mice were used. 129X1/SvJ. The 129X1/SvJ inbred mouse strain originated from crosses established in 1928. It is the strain most commonly used to generate ES cells for gene knockout studies (22).

Materials and Methods

Mice

CD-1 mice were purchased from Charles River Breeding Laboratories (Wilmington, MA); all other strains where purchased from The Jackson Laboratory (JAX mice; Bar Harbor, ME). The animals were housed in Jag-75 plastic cages with wood shavings in a high efficiency particulate air-filtered barrier unit maintained at 25°C with alternating 12-h periods of light and dark. The mice had access to drinking water at all times and were fed ad libitum with an autoclaved pelleted rodent diet. The great majority of mice used were between 6 and 12 wk of age at the time of the experiment. Four strains of mice were used. 129X1/SvJ. The 129X1/SvJ inbred mouse strain originated from crosses established in 1928. It is the strain most commonly used to generate ES cells for gene knockout studies (22).

C57BL/6J. C57BL/6d is widely used, accounting for 14% of all occasions in which an inbred strain has been used in the literature (http://www.informatics.jax.org).

BALB/c. The BALB/c inbred mouse strain is commonly used in the field of immunology (23).

CD1. CD1 is a commonly used albino, outbred mouse strain originating from Switzerland.

Thioglycolate medium

Two variants of TG were tested: ready-to-use, aged TG from stored at 4°C (BD Bioscience, Franklin Lakes, NJ), and dehydrated Brewer TG medium (Difco, Detroit, MI). The latter was taken up as a 3% solution (30 g of TG powder in 1000 ml of deionized water) and was autoclaved 20 min at 15 lbs/psi (121°C). The sterile 3% TG was protected from light and was “aged” at room temperature for at least 1 mo to ensure formation of advanced glycation end products (20). Preprepared and laboratory-prepared TG were identical in their abilities to trigger peritoneal recruitment of macrophages in all four mouse strains studied (data not shown). All data presented in this report were obtained through the use of preprepared TG medium.

Peritoneal cell recruitment

For recruiting peritoneal cells, 1 ml of sterile TG medium (warmed to room temperature) was injected into the peritoneal cavity of male or female mice using 23-gauge needles. For neutrophils, harvesting of peritoneal cells 4 h post-TG injection has been recommended for optimal yield (19). For macrophages, harvesting of peritoneal cells 3–5 days post-TG injection has been shown yield optimal numbers (20, 21, 24). After the appropriate amount of time, mice were CO₂ asphyxiated and cells were harvested by intraperitoneal lavage. Seven milliliters of ice-cold Dulbecco’s PBS (DPBS) in a 10-ml syringe with a 23-gauge needle was injected into the lower left abdominal peritoneal cavity, close to the left iliac fat pad. Injection was conducted through the fat pad because this approach seals the puncture hole, preventing loss of DPBS-containing cells. The peritoneal compartment was gently massaged and agitated for 30 s, then the gut or membrane was ruptured, the sample was discarded. Using this method, recovery was consistently 6.7 ± 0.6 ml. If at any point the gut or membrane was ruptured, the sample was discarded and the white blood cell pellet was resuspended in 1 ml of DPBS for immediate counting. Duplicate 10-μl samples were counted manually using a hemocytometer. The average of the two counts was recorded as the total number of cells harvested from that mouse. Based on the total cell count, 1 × 10⁵ cells were suspended in 200 μl of DPBS containing 4% BSA. The entire 200 μl was then loaded into a cytopsin chamber and centrifuged at 500 × g for 5 min at room temperature in a cyto-centrifuge. Slides were Wright-Giemsa stained (Diff-Quick; Dade Behring, Newark, DE) and differential cell counts were performed.

Statistical analysis

Significance of differences were tested using Student’s paired t test. Probabilities were considered significant at the 5%, 1%, and 0.1% levels. Results are expressed as mean values ± SEM.

Results

Analysis of cell recruitment in DBP-null mice

A previously described DBP-null (DBP−/−) mouse model (9) was used to evaluate the in vivo role of DBP in an established leukocyte recruitment model. An established model of cell recruitment to a site of chemical inflammation was used for this analysis. Aged TG broth was injected into the peritoneal cavity of the mouse and the subsequent recruitment of inflammatory cells was quantified at defined time points. A comparison was conducted between the responses in DBP−/− and DBP-positive (DBP+/+) or wild-type (WT) mice. The pilot studies used DBP−/− and DBP+/+ mice with mixed genetic backgrounds that had been generated in the following manner. The DBP gene deletion was generated by homologous recombination in 129X1/SvJ-derived ES cells (R1; Ref. 25) and the selected ES clone was injected into C57BL/6d blastocysts. The chimeric progeny of these injected blastocysts were mated to CD1 mice to generate the F₁ generation. F₁(DBP+/−) mice were then intercrossed to generate DBP−/− mice. To mini-
mize the need for repeated genotyping, the DBP−/− mice were generated by DBP−/− × DBP−/− matings to maintain a DBP−/− stock for analysis. In parallel, DBP+/+ controls were generated by parallel mating to CD1 outbred mice. Thus, the initial series of studies was conducted with DBP−/− and DBP+/+ mice that were on mixed genetic backgrounds of undetermined relationship to each other due to this period of segregating mating. Mice from the DBP−/− and DBP+/+ groups were injected with TG and the peritoneal cells were harvested 4 days later (Materials and Methods). There was a 10-fold difference (p < 0.001) in the cell recruitment between the two groups (Fig. 1A, Mixed). In fact, there was no significant recruitment in the DBP−/− mice at this time point; the total numbers of cells in the peritoneal cavity of the TG- and saline-injected DBP−/− mice at 4 days were equivalent. In these pilot studies, the data thus demonstrated a total and selective inability of the DBP−/− mice to recruit cells to the peritoneum in response to TG.

Immune functions can be dictated by complex genetic interac-
tions. For this reason, we next determined the extent to which DBP contributed to the observed difference in cell recruitment and to what extent the observed phenotype reflected uncontrolled genetic differences between the DBP−/− and WT test groups. To most clearly define the unique contribution of DBP to the recruitment effect, the DBP-null locus was bred into a homogenous genetic background, and WT littermates were used as controls. Because C57BL/6d is the most widely used inbred mouse strain and is typically used as the background strain for induced mutations, a series of outcrosses of the DBP-null line with the C57BL/6d isogenic line was conducted. These outcrosses were conducted as follows: DBP−/− × C57BL/6d matings produced DBP−/− heterozygous progeny (100%). The heterozygous progeny were then inter-
crossed to produce outcross 1 (OX1) pups: DBP−/− (25%),
Saline injections. Details as in C57BL/6J were analyzed for cell recruitment 4 days post-TG or control recruitment when compared with the C57BL/6J strain (data not shown). The number of animals in each group is shown below the corresponding set of histogram bars. The significance of differences between recruitment within each pair is indicated (***, p < 0.001). A, 129X1/SvJ mice have a profound defect in cell recruitment when compared with the C57BL/6J strain. Pure 129X1/SvJ and C57BL/6J mice were analyzed for cell recruitment 4 days post-TG or control saline injections. Details as in A.

The loss of the apparent DBP-mediated defect in inflammatory cell recruitment during the outcrossing to the C57BL/6J strain and the corresponding overall increase in cellular recruitment suggested that the defect originally detected might be related to the 129X1/SvJ genetic background. To confirm and extend this observation, we directly compared the TG-stimulated recruitment potential in the 129X1/SvJ and C57BL/6J isogenic strains (Fig. 1B). Four days after TG stimulation, the C57BL/6J strain responded with a 12-fold increase in peritoneal cells over saline-injected controls compared with a total lack of increase in the 129X1/SvJ strain (p < 0.001). Thus, the 129X1/SvJ line demonstrated a marked defect in inflammatory cell recruitment.

To formally determine whether the difference in the cell recruitment in the C57BL/6J strain and the 129X1/SvJ strain reflected the action of a single dominantly acting “cell recruiter” gene, the two isogenic strains were crossed and cell recruitment was measured in the F1 generation. These F1 mice, obligate heterozygotes for 129X1/SvJ and C57BL/6J alleles at all loci, demonstrated a recruitment level that was midway between the two parental strains (data not shown). Thus, the observed recruitment phenotypes cannot be explained solely on the simple basis of a dominantly acting cell recruiter gene. Such a situation would have resulted in all F1 mice having a recruitment phenotype equivalent to that of the C57BL/6J strain. Therefore, the difference in cell recruitment between the C57BL/6J and 129X1/SvJ strains must reflect codominant or more complex genetic mechanisms.

Complex genetic regulation of the 129X1/SvJ recruitment defect: distinct levels of peritoneal cell recruitment in four commonly used mouse strains

To further define the relationship of cell recruitment to mouse strain, we analyzed the recruitment response to TG in four of the most commonly used mouse strains: isogenic strains C57BL/6J, BALB/c, and 129X1/SvJ, and outbred CD1. Mice from each strain were injected with 1 ml of 3% TG, and peritoneal cells were harvested after 4 days. The total cell recruitment in each strain was strikingly different (Fig. 2A); C57BL/6J was the most responsive, followed by BALB/c, CD1, and the least responsive was the 129X1/SvJ strain. All pairwise comparisons revealed highly significant differences (p < 0.001). Differential cell counts were established on the recruited cells in each strain (Fig. 2B). These data revealed that macrophages formed the majority of the cells recruited to the peritoneum after 4 days (70–90%). Macrophage numbers closely paralleled the total cell counts.

Distinct strain-related recruitment kinetics

To characterize the difference in recruitment phenotypes in greater detail, a time course of cell recruitment was conducted in the C57BL/6J and 129X1/SvJ strains (Fig. 3, A–C). Twenty-eight mice of each strain were injected with TG, and peritoneal cells from four mice of each strain were harvested at 0 and 4 h postinjection and on days 1, 2, 3, 4, and 5. Total and differential cell counts were determined at each time point. Before injection with TG, resident cell counts were similar in the two strains. At 4 h post-TG injection, the total cell counts increased 2- to 3-fold in both strains. Of note, the majority of cells in both groups at 4 h were neutrophils. Macrophages did not appear in significant numbers in either group until day 1. In the 129X1/SvJ strain, the total cell counts were at their highest 4 h post-TG injection, and fell progressively at subsequent time points. In contrast, the total cell count in the C57BL/6J strain rose steadily from 4 h through 2 days, plateaued from 2 to 4 days, and then began to decrease on day 5. During this period, the differential cell count of the cell population...
in the C57BL/6J peritoneal fluid shifted from predominantly neutrophils at 4 h and at 1 day post-TG injection to an increasingly predominant proportion of macrophages at 2, 3, 4, and 5 days (70%, 85%, 97%, and 99%). A similar shift from neutrophils to macrophages was seen in the 129X1/SvJ mice, although overall cell numbers were much lower and the levels dropped earlier. During the entire time course, a small number of lymphocytes were also seen; the absolute number of lymphocytes was low at all times and showed a higher degree of variability (data not shown). Thus, there was an equivalent recruitment of neutrophils in both groups in response to TG at early time points postinjection. The major and sustained macrophage response seen in the C57BL/6J mice did not occur in the 129X1/SvJ mice. Thus, the defect in inflammatory cell recruitment is not global, but appears to be limited to the macrophage/monocyte lineage.

**Peritoneal neutrophil recruitment in DBP<sup>−/−</sup> mice**

There was no defect in peritoneal monocyte/macrophage recruitment attributable to DBP because no significant differences in cell counts at 4 days postinjection in OX2 through OX4 generations were observed (Fig. 1A). Although strain-related leukocyte recruitment kinetics differed markedly, the absolute level of neutrophil recruitment was not significantly different in C57BL/6J and 129X1/SvJ mice at 4 h after TG injection (Fig. 3C). Therefore, to determine whether the DBP allele contributed to peritoneal neutrophil recruitment, cells from DBP<sup>−/−</sup> and DBP<sup>+/+</sup> mice from OX3 were studied at 4 h post-TG injection. Mean and SEM neutrophil counts in DBP<sup>−/−</sup> and DBP<sup>+/+</sup> groups were (11.4 ± 1.47) x 10<sup>6</sup> and (8.8 ± 0.98) x 10<sup>6</sup> cells/ml, respectively (n = 5). These differences were not significant (p = 0.163). Thus, no significant role could be attributed to DBP in either the macrophage or neutrophil recruitment in this experimental model.

**Discussion**

The goal of this study was to determine whether DBP serves a unique and nonredundant role in innate immune function. We assessed the ability of a recently established DBP-null mouse line to recruit macrophages and neutrophils to a localized site of TG-induced sterile inflammation. In line with this aim, the initial data revealed a profound defect in macrophage recruitment in the DBP<sup>−/−</sup> mice. However, progressive outcrossing of the mDBP<sup>−/−</sup>
mice onto a pure C57BL/6J background resulted in a progressive increase in the ability of the DBP-null mice to recruit inflammatory cells. Further analysis confirmed that the initially observed deficit in the cellular response was not linked to the DBP-locus, but instead correlated with the 129X1/SvJ genetic background of the mice being studied. Comparisons of macrophage recruitment among four commonly used mouse strains revealed differences in the numbers of macrophage recruited after 4 days in each strain: C57BL/6J > BALB/c > CD1 > 129X1/SvJ. Genetic crosses between 129X1/SvJ and C57BL/6J mice further supported the presence of a quantitative recruitment trait. We conclude that DBP does not contribute a unique function to neutrophil or macrophage recruitment in the context of the present experimental model. Rather, these studies revealed that the 129X1/SvJ strain has a major defect in its ability to recruit macrophages to the peritoneal compartment.

The observed defect in cell recruitment in the 129X1/SvJ strain was unanticipated and would predict an increased susceptibility to certain infectious diseases. This prediction is supported by two observations. In a study by others of the resistance of different mouse strains to Mycobacterium tuberculosis infection, 129X1/SvJ mice were found to be highly susceptible, whereas C57BL/6J mice were highly resistant to infection (27). Significantly, clearance and killing of M. tuberculosis is a macrophage-dependent process, and the observed defect in resistance to M. tuberculosis infection parallels the defect in macrophage recruitment to TG-induced inflammation detected in the present study. A set of data from our own laboratory further supports the significance of the current study. We have observed a marked decrease in the rate of in vivo clearance of the intracellular parasite Leishmania donovani in “mixed” genetic background DBP+/− mice (P. White, M. Murphy, S. Liebhaber, J. Farrell, and N. Cooke, manuscript in preparation). Remarkably, this defect in L. donovani clearance is lost upon outcrossing the DBP−/− allele to the C57BL/6J strain. Thus, observations regarding resistance to M. tuberculosis and L. donovani suggest that the cell recruitment defect identified in the 129/SvJ strain using the TG-stimulated inflammation model reflects physiologically relevant defects in macrophage recruitment and/or function.

The observed contribution of the 129X1/SvJ genome to the phenotype of the DBP−/− mice in the initial analysis (Fig. 1A, Mixed) can be traced back to the fact that the DBP gene mutation was originally introduced in 129X1/SvJ ES cells. These cells were then injected into a C57BL/6J blastocyst. The subsequent mating of the chimera founder mouse to a C57BL/6J mouse resulted in DBP−/− progeny that carried one set of 129X1/SvJ genes and one set of C57BL/6J genes. Interbreeding these DBP+/− mice was conducted to generate progeny that were homozygous null, heterozygous null, and WT at the DBP locus. These mice had recombinant genotypes from the two parental strains; certain loci (including the disrupted DBP allele) were derived from 129X1/SvJ strain and others from C57BL/6J strain. Thus, the WT littermates did not constitute an optimal control population for the DBP−/− mice because the non-DBP alleles in each mouse differed. Whereas expanding the population size under study would provide statistical power to minimize this problem, this approach is not able to fully randomize the alleles. Furthermore, genes closely linked to the DBP locus would be unlikely to segregate from the disrupted DBP allele and would therefore remain largely of 129X1/SvJ origin. This was of particular relevance in the present situation because peritoneal leukocyte recruitment is considered to be dependent upon local chemokine gradients (28), and the human DBP gene is closely linked to a cluster of nine CXC chemokine genes, including IL8 and the GRO1, GRO2, and GRO3 genes (29, 30). Significantly, the defect in cell recruitment in the DBP−/− mice in the initial analysis was indistinguishable from that seen in the 129X1/SvJ strain (Fig. 2A). The recruitment profiles in the DBP−/− and DBP+/− groups were indistinguishable from each other after several rounds of outcrossing of these DBP−/− mice to the C57BL/6J strain. These data are fully consistent with the conclusion that the initial recruitment defect in the DBP−/− mice reflected their 129X1/SvJ genetic content and not DBP or a DBP-linked CXC chemokine gene.

What is the genetic basis for the recruitment defect in the 129X1/SvJ mice? The intermediate level of recruitment in the F1 progeny of the 129X1/SvJ and C57BL/6J intercross (see Results) demonstrated that the inflammatory response was not controlled by a single dominantly acting “recruiter locus.” Three inbred lines have three distinct levels of recruitment (Fig. 2), allowing us to further conclude that the trait is controlled by three or more alleles at a single locus or that the trait is polygenic. The graded levels of recruitment with each outcross of the initial mouse line to the C57BL/6J strain (Fig. 1A) further supports the conclusion that the trait is the product of a complex set of determinants that, in sum, result in a net recruitment level. Definitive assignment of the genetic basis for the distinct cell recruitment phenotypes will of course be based on subsequent genetic and biochemical mapping studies.

Our examination of cell recruitment over a 5-day period revealed strain-specific effects in the kinetics of leukocyte recruitment. Previous reports indicated that peak recruitment of peritoneal neutrophils was observed 4 h post-TG injection (19). We found this to be true for 129X1/SvJ, but the more robust neutrophil response in C57BL/6J occurred later (24 h). Because the neutrophil recruitment remained essentially intact except for this kinetic shift, it is likely that generally acting, receptor-linked chemoattractant pathways and the actin cytoskeletal structures remain intact. Mutant genes in such pathways would be expected to result in global defects in the inflammatory cell recruitment phenotype, and the defect observed was remarkably limited to the macrophage/monocyte lineage. For macrophages, the peak response has typically been reported to occur 3–5 days post-TG injection in studies using Sprague Dawley rats, Swiss mice, or C3H/HeN mice (20, 21, 24). In contrast, the few macrophages that were recruited to the peritoneum in the 129X1/SvJ strain appeared much earlier (2 days). The kinetic differences for these two cell types, as well as the distinct quantitative responses in the various inbred lines, are consistent with a complex genetic basis of the overall inflammatory cell recruitment process.

The results of this current study highlight the need for careful outcrossing to establish reliable control groups for targeted knock-out lines before phenotypic characterization. This is of particular importance in dealing with complex genetic phenotypes such as those involving the immune system. The present study emphasizes that this need is of specific importance in the analysis of peritoneal leukocyte recruitment phenotypes. In a survey of the literature, we have identified a set of published papers in which a peritoneal recruitment defect in response to TG has been attributed to the targeted interruption at each of four specific loci. These loci include the CCR-2 gene (31, 32), the monocyte chemoattractant protein-1 gene (33), the plasminogen gene (34), and the Duffy gene (35). It is noteworthy that in each case, the gene disruption was introduced by homologous recombination into 129 ES cells that were implanted in a C57BL/6J blastocyst, as in the current study. In addition, in each case, the 129X1/SvJ × C57BL/6J mixed genetic background mice homozygous for the null allele were demonstrated to have 2- to 10-fold lower cell counts than WT controls 3–4 days after i.p. TG injection, whereas the 4-h neutrophil counts
were unaffected. These results are remarkably similar to those observed in our initial analyses of the DBP−/− mice obtained on mixed genetic backgrounds before outcrossing to the C57BL/6J (Figs. 1 and 3C). Of note, in none of these studies were backcrosses into a purebred strain undertaken to be certain that the recruitment phenotype reflected the biologic effect of the gene knockout as opposed to a genetic background effect of the 129X1/SvJ strain. In light of our present finding that the 129X1/SvJ strain has a profound deficit in macrophage recruitment, it is reasonable to suggest that genotype-phenotype conclusions based on observations in these studies, and others like them, will require careful reconsideration. In addition, the demonstrated strain-dependent differences in the cell recruitment process may be used to advantage in future studies to dissect the various genetic determinants of this innate immune function.

Acknowledgments

We thank Dr. Fayez Safadi for early discussions, Ivona Percec for a careful reading of the manuscript, and Dr. Richard Spielman for critical comments.

References

2. Wolfer, D. P., and H. P. Lipp. 2000. Dissecting the behaviour of transgenic mice: is it the mutation, the genetic background, or the environment? Exp. Physiol. 85:627.