DIFFERENCES IN THE RESPONSE OF INBRED MOUSE STRAINS TO THE FACTOR INCREASING MONOCYTOPOIESIS

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Inflammatory reactions to various kinds of stimuli are characterized by an increase in the number of macrophages derived from circulating monocytes at the site of inflammation and by a concomitant monocytosis (1–3). The increase in the number of macrophages is brought about by an increased monocyte production in the bone marrow, which occurs by augmentation of the rate of division of the monoblasts and promonocytes (4). This process is regulated in mice (3) and rabbits (5) by a humoral factor called the factor increasing monocytopoiesis (FIM).[1] FIM is a protein that is synthesized and secreted by macrophages at the site of inflammation and then transported via the circulation to the bone marrow, where it exerts its stimulatory action (6, 7). The biological activity and physicochemical characteristics of FIM differ from those of colony-stimulating factor (CSF) (5, 6). The increase of the number of macrophages in the inflammatory exudate in response to a variety of stimuli has been shown to be controlled by a single, autosomal, dominant gene (8). The mechanism underlying the genetic control of the magnitude of the inflammatory reaction remains to be elucidated.

Since monocyte production during an acute inflammation is stimulated by FIM, which in turn gives rise to increased numbers of macrophages in the inflammatory exudate, it seemed possible that high and low responder mouse strains differ with respect to the production of FIM or the sensitivity of their monocyte precursor cells for this protein. This paper reports a study performed to discover whether the humoral regulation of monocyte production is genetically determined.

Materials and Methods

Animals

Specified pathogen-free, female CBA/Rij (H-2^k) mice (REPGO-TNO, Rijswijk, The Netherlands) and C57BL/10ScSnCpb (H-2^b) mice (The Central Institute for the Breeding of Laboratory Animals, TNO, Zeist, The Netherlands) were used at 6–12 wk of age. All

This work was supported in part by the Nederlands Astma Fonds.

[1] *Abbreviations used in this paper*: AUC, area under curve; FCS, fetal calf serum; FIM, factor increasing monocytopoiesis; LaS, serum from mice injected intraperitoneally with latex; SEL, saline extract of *Listeria monocytogenes*; SELS, serum of mice injected intraperitoneally with SEL.

 J. EXP. MED. © The Rockefeller University Press · 0022-1007/84/02/0524/13 $1.00
Volume 159 February 1984 524–536

mice were kept singly in cages and allowed to acclimate to the new environment (5). In each experiment, the animals were matched for age and handled in a random order. Sterilized food and tap water were given *ad libitum.*

Induction of an Acute Inflammation

Latex. An acute inflammation was induced by intraperitoneal injection of ~2.5×10^8 latex particles (Bactolatex, 0.81 μm diam; Difco Laboratories, Detroit, MI) in 1 ml of sterile, pyrogen-free saline. This suspension contained <20 pg endotoxin, as assayed with the *Limulus* amebocyte lysate (LAL) gelation test (9) (kindly performed by Dr. J. G. Kreeftenberg, Rijks Instituut voor de Volksgezondheid, Bilthoven, The Netherlands).

Saline extract of Listeria monocytogenes. A saline extract of *L. monocytogenes* (SEL) serotype 1 (kindly provided by Dr. I. J. M. A. Bakker-Woudenberg, Erasmus University, Rotterdam, The Netherlands) was prepared as described by Galsworthy et al. (10) with an additional extraction with 0.1 M NaCl. The saline-solute material was dialyzed against distilled water for 24 h, lyophilized, dissolved in pyrogen-free saline at a concentration of 10 mg/ml, passed through a 0.45 μm Millex-HA filter unit (Millipore, Molsheim, France), and stored in small aliquots at −20°C in sterile pyrogen-free tubes (nontoxic plastic; Falcon Labware, Oxnard, CA). This preparation contained <5 pg endotoxin per mg SEL (spectrophotometric lipopolysaccharide assay using chromogenic substrate S2422 [11], kindly performed by Dr. L. L. M. Thomas, University Medical Centre, Amsterdam, The Netherlands). An inflammatory reaction was evoked by injection of 1 mg SEL intraperitoneally.

Harvesting and Counting of Peritoneal Leukocytes

Peritoneal cells were harvested under strict standard conditions, as described in detail elsewhere (2), with exactly 1.5 ml phosphate-buffered saline, pH 7.2 (Difco Laboratories), containing 50 U/ml heparin. The cells were counted in duplicate in a Bürker hemocytometer and characterized in Giemsa-stained cytocentrifuge preparations. The numbers of peritoneal cells were expressed per milliliter wash-out fluid since the total numbers could not be determined.

Counting of Peripheral Blood Leukocytes

Peripheral blood was obtained by puncture of the orbital plexus with a 50 μl capillary (Accupette; Dade Diagnostics, Miami, FL) and diluted with an equal volume of heparin. After further dilution in Isoton II (Coulter Electronics GmbH, Krefeld, Federal Republic of Germany) and lysis of the erythrocytes by addition of Lyse S (Coulter Electronics), leukocyte counts were performed in quadruplicate with a Coulter Counter model ZF (Hoek-Loos, Schiedam, The Netherlands). The total numbers of monocytes, granulocytes, and lymphocytes were calculated from the total number of leukocytes and differential counts of at least 400 leukocytes in four Giemsa-stained blood smears, and were expressed per milliliter of blood.

Preparation of Serum

The preparation of serum has been described in detail elsewhere (3).

Assay of FIM Activity

In vitro assay. For the assessment of FIM activity, cells of the macrophage cell line PU5-1.8 (kindly donated by Dr. P. Ralph, Sloan-Kettering Institute for Cancer Research, Rye, NY), recovered from a log-phase culture in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) containing 10% fetal calf serum (FCS), 2 mM L-glutamine, 1,000 U/ml penicillin, and 50 μg/ml streptomycin were incubated in triplicate at a concentration of 2×10^5/ml culture medium that, instead of 10% FCS, contained 8% FCS and 2% of the mouse serum under study. After overnight culture in Teflon culture bags (12) in a humidified atmosphere of 7.5% CO_2 in air at 37°C, the cells were counted and cell viability was assessed by trypan blue exclusion. The mean number of viable cells grown in culture medium containing 10% FCS was used as reference value (N_c). The FIM activity

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index (I) was calculated as the percentage increase of the number of cells in cultures with serum under investigation (N_s) in relation to the reference value (N_c), according to the formula: $I = (N_s/N_c - 1) \times 100$.

In vivo assay. The ability of serum to induce monocytosis was assayed in normal test mice by determination of the number of blood monocytes in each animal at 24 h before and 24, 48, and 72 h after the intravenous injection of 0.2 ml of a serial twofold dilution in pyrogen-free saline. Since the number of blood monocytes in normal mice, whose blood is sampled only once a day for a leukocyte count, remains constant over a period of several days (manuscript in preparation), the time point 24 h before the serum injection was taken as time zero. Under these conditions, the animals were stressed only once a day.

The monocytosis-inducing activity was expressed as the difference (Δ_{24}) between the number of blood monocytes in mice at time zero and at 24 h after injection of a serum sample (monocytes $\times 10^{-6} \times ml^{-1}$) and also as the area under the curve (AUC) of the number of blood monocytes during 72 h after injection of the sample (monocytes $\times 10^{-6} \times h \times ml^{-1}$) calculated according to the formula, $AUC = {}_0\int^t N dt - N_0 t$, in which N is the number of blood monocytes $\times 10^{-6}$/ml and t is the duration of the observtion period (i.e., 72 h).

Statistical Analysis

In all in vivo experiments, at least four but usually five mice were used per time point. The in vitro experiments were performed in triplicate and the results expressed as the mean and standard error of the mean. To assess the significance of differences within experiments, we used Student's two-tailed test or the analysis of variance (13). Unless stated otherwise, all P values mentioned refer to differences between treated and control mice or cultures. The extent to which the leukocyte response was influenced by a number of variables (considered in totality, 41) was assessed by covariance analysis with an IBM 370/158 computer using the stepwise procedure in a multiple regression analysis (14). The actual decisions as to whether a particular variable must be added to or removed from the multiple regression equation were based on P-to-enter and P-to-remove values, which were set at $\leqq 0.05$ and $\geqq 0.10$, respectively. For the regression analyses, the following values were assigned arbitrarily in advance to the variable inflammatory agent: 0 (zero) for SEL and 1 for latex; for donor mouse strains, 0 (zero) for CBA and 1 for B10 mice; and for the recipient strains, 0 (zero) for CBA and 1 for B10 recipients.

Results

Number of Blood and Peritoneal Leukocytes in Normal B10 and CBA Mice

The numbers of leukocytes in the peripheral blood and peritoneal cavity were determined in B10 and CBA mice to obtain the normal values for these mouse strains. The results show a slight but statistically significant difference in the number of blood monocytes between the two strains; small differences were also found between the numbers of granulocytes and lymphocytes of both strains (Table I). The number of macrophages in the peritoneal cavity did not differ significantly between the two strains, but CBA mice had twice as many peritoneal lymphocytes as B10 mice and half the number of peritoneal granulocytes (Table I).

Course of Mononuclear Phagocyte Numbers in Response to Intraperitoneally Injected Sterile Inflammatory Agents

To compare the magnitude of the cellular reaction in B10 and CBA mice, an inflammation was induced in the peritoneal cavity by injection of a sterile particulate or soluble inflammatory stimulus.

Peritoneal macrophages. After an intraperitoneal injection of polystyrene latex

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TABLE I
Pool Sizes of Leukocytes of B10 and CBA Mice

	Number of leukocytes ($\times 10^6$/ml) in mice of strain:		Difference between B10 and CBA
	B10	CBA	
			P
Peripheral blood*			
Monocytes	0.25 (±0.07)	0.19 (±0.05)	<0.0005
Granulocytes‡	0.57 (±0.23)	0.70 (±0.24)	<0.0005
Lymphocytes	7.55 (±1.57)	8.05 (±1.48)	0.025
Peritoneal cavity§			
Macrophages	2.09 (±0.48)	2.18 (±0.47)	NS
Granulocytes	0.03 (±0.02)	0.01 (±0.03)	0.005
Lymphocytes	0.07 (±0.05)	0.15 (±0.07)	<0.0005

* Values are means of 95 mice and the standard deviation.
‡ Marginating pool is not taken into account.
§ Values are means of 30 mice and the standard deviation.

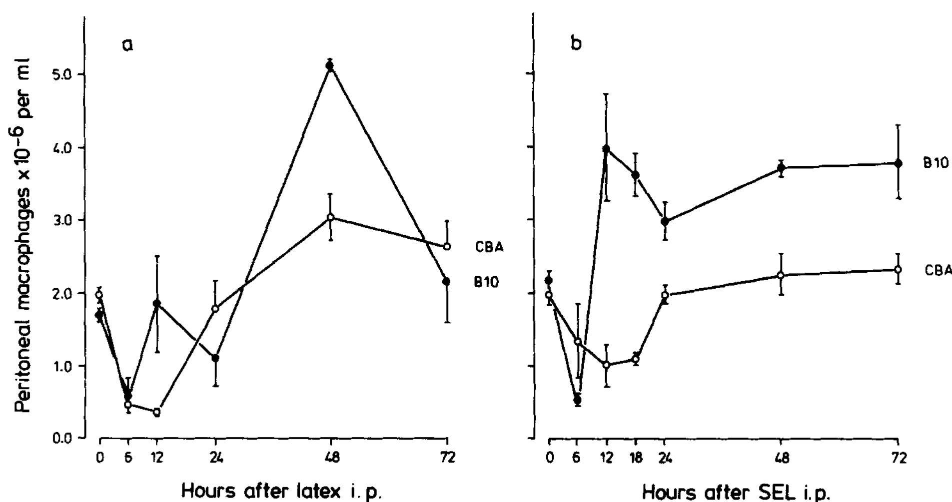

FIGURE 1. Course of the number of macrophages in the peritoneal cavity of C57BL/10 mice (●) and CBA mice (○) after an intraperitoneal injection of 1 ml of a latex suspension (*a*) or 1 mg SEL (*b*).

particles into B10 mice, the number of peritoneal macrophages first decreased to a minimum at 6 h ($0.01 < P < 0.02$) and then increased to a maximum of 5.10 (±0.07) $\times 10^6$/ml at 48 h ($P < 0.001$), followed by a return to normal values at 72 h (Fig. 1 *a*). In the peritoneal cavity of CBA mice, the number of macrophages decreased after injection of latex to a minimum at 12 h ($P < 0.001$) and then increased slightly to a maximum of 3.02 (±0.32) $\times 10^6$/ml at 48 h ($0.01 < P < 0.02$) (Fig. 1 *a*).

After injection of SEL into B10 mice, the number of peritoneal macrophages fell off to a minimum of 0.52 (±0.08) $\times 10^6$/ml at 6 h ($P < 0.001$), then increased

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rapidly to a maximum of 3.96 (±0.97) × 10^6/ml at 12 h ($0.02 < P < 0.05$), and remained at roughly that level over the next 60 h (Fig. 1*b*). In CBA mice, the number of peritoneal macrophages also showed a decrease after injection of SEL, and did not return to normal values until 24 h after injection, but then increased slightly to a maximum of 2.33 (±0.20) × 10^6/ml at 72 h ($0.1 < P < 0.2$) (Fig. 1*b*).

These results show that, for all inflammatory stimuli used, the accumulation of peritoneal macrophages was greater in B10 than in CBA mice. The difference between the AUC amounted for both stimuli to a factor of ~1.6.

Blood Monocytes

After the intraperitoneal injection of latex, the number of blood monocytes of B10 mice reached a maximum of 7.09 (±1.02) × 10^5/ml at 24 h ($0.01 < P < 0.02$) and then subsided to normal values at 72 h (Fig. 2*a*). In the peripheral blood of CBA mice, the number of monocytes increased rapidly to a maximum of 3.35 (±0.55) × 10^5/ml at 6 h after latex injection ($0.02 < P < 0.05$), then decreased over the next 12 h, followed by a slight increase (Fig. 2*a*)

After the injection of SEL, the number of blood monocytes in B10 mice rose to a maximum of 3.75 (±0.42) × 10^5/ml at 18 h ($0.01 < P < 0.02$) and then returned to the normal level over the next 30 h (Fig. 2*b*). The course of the number of monocytes in the peripheral blood of CBA mice remained fairly constant after the injection of SEL, except for a statistically significant decrease at 6 h ($0.01 < P < 0.02$) (Fig. 2*b*). For latex and SEL, the difference between the AUC amounted to factors of 2.2 and 1.7, respectively, in favor of the B10 mice.

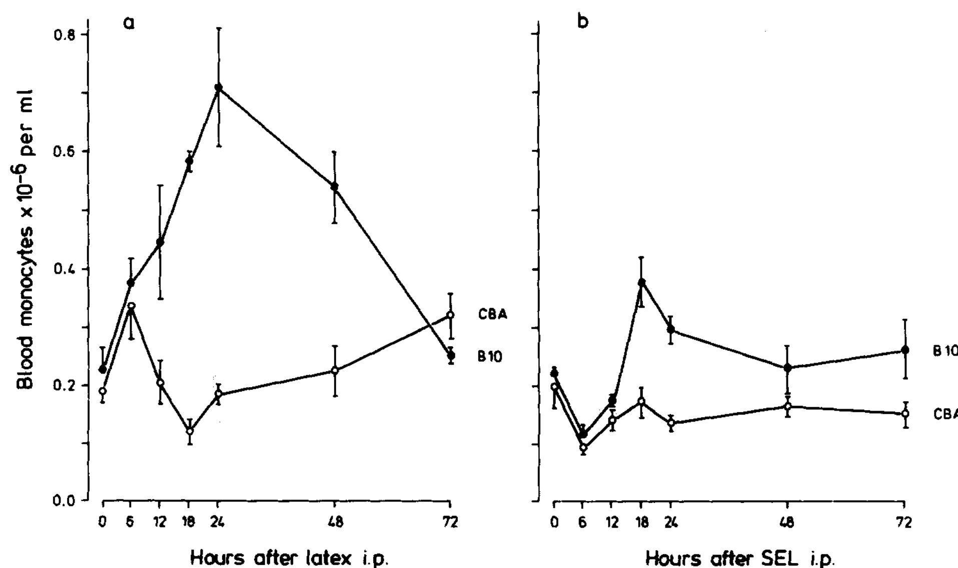

FIGURE 2. Course of the number of monocytes in the peripheral blood of C57BL/10 mice (●) and CBA mice (○) after intraperitoneal injection of 1 ml of a latex suspension (*a*) or 1 mg SEL (*b*).

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Demonstration of FIM Activity in Serum

To find out whether the difference in inflammatory response between B10 and CBA mice expressed in the number of macrophages and blood monocytes was based on a difference in FIM production, the serum level of this protein was determined by in vitro assay at various intervals after intraperitoneal injection of the inflammatory stimulus.

FIM activity was demonstrable in the serum of both B10 and CBA mice given latex intraperitoneally; although differences were found between the two strains with respect to the course of FIM activity after the injection of latex, the maximum levels were almost the same (Fig. 3*a*).

After the intraperitoneal injection of SEL, the serum of the two mouse strains showed almost synchronous courses of FIM activity and similar peak values (Fig. 3*b*). To rule out the possibility that these assay results were influenced by trace amounts of SEL in the serum, 20 μg SEL was added to the cultures of the cell line, under the assumption that all intraperitoneally injected SEL had been diffused to the blood compartment. The index was −10.4 (Fig. 3*b*), which is significantly lower ($0.02 < P < 0.05$) than the values obtained when normal B10 or CBA serum was added (FIM indices, 4.8 and −3.2, respectively.)

Relationship Between the Dose of Inflammatory Stimulus and FIM Activity in Serum

To determine whether the index of FIM activity is related to the dose of inflammatory stimulus, serial twofold dilutions of latex in saline were injected intraperitoneally, and blood samples were taken at time points when maximal activity would be expected (i.e., for B10 mice at 24 h and for CBA mice at 12 h) for in vitro assays of FIM activity in serum. The results showed that in both strains of mice the index of FIM activity increased with decreasing dilutions of latex until a maximum was reached at the dose normally given intraperitoneally (i.e., the stock suspension containing 2.5×10^8 particles/ml); however, when

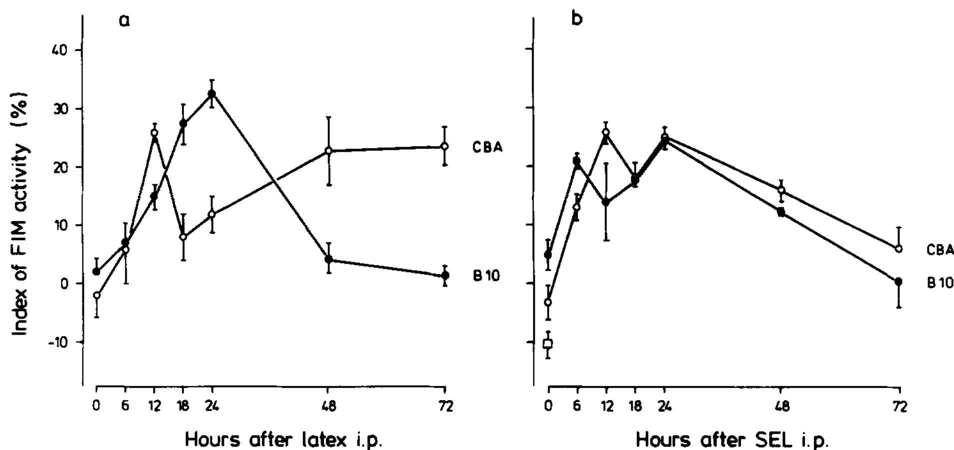

FIGURE 3. Course of the index of FIM activity in serum of C57BL/10 mice (●) and CBA mice (○) at various time points after intraperitoneal injection of 1 ml of a latex suspension (*a*) or 1 mg SEL (*b*), determined by an in vitro bioassay (see Materials and Methods). The effect of 20 μg of SEL alone (□) is also shown in *b*.

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double this concentration of latex particles were administered, the index was considerably lower than was found with stock suspension (Fig. 4).

For a simple mathematical analysis of the relation between FIM activity in serum and the dose of latex given intraperitoneally, the $\log_2$ dose interval from −2 to 0 (zero) was considered (Fig. 4). The regression of the index of FIM activity (I) on the $\log_2$ dose of latex (dose) can be described for B10 mice by the equation:

$$I = 17.5 + 7.1 \text{ dose } (r = 0.79),$$

and for CBA mice by:

$$I = 19.5 + 11.7 \text{ dose } (r = 0.95).$$

Analysis of the covariance of these regression lines for homogeneity showed no significant differences in their residual variances ($0.10 < P < 0.25$) and slopes ($0.05 < P < 0.10$), but their average slope (b) was found to be highly significant (b = 9.4; $0.001 < P < 0.005$). This means that statistically these regression lines run parallel. The computed intercepts of the two regression lines with the y axis did not differ significantly ($0.10 < P < 0.25$), which means that the relationship between the index of FIM activity and the amount of latex injected intraperitoneally was identical for the two mouse strains.

In Vivo Response to Serum Containing FIM

The effect of serum with FIM activity on the number of circulating leukocytes was studied next. For this purpose, B10 and CBA mouse sera with maximal activity, as determined in the in vitro bioassay (Fig. 3*a* and *b*), were injected intravenously into normal B10 and CBA recipient mice in serial twofold dilutions in saline. The effect was expressed as the difference between the number of

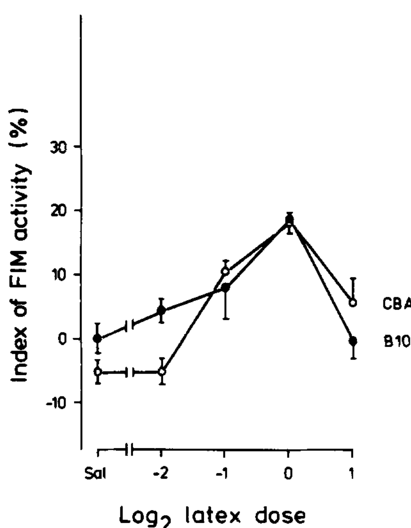

FIGURE 4. Index of FIM activity in serum of C57BL/10 mice (●) at 24 h and of CBA mice (○) at 12 h after injection of 1 ml of serial twofold dilutions of latex in saline. The stock suspension, whose $\log_2$ value was put at zero, contained 2.5×10^8 particles.

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leukocytes at time zero and at 24 h after injection (Δ_{24}) or as the AUC from time zero to 72 h.

In B10 recipient mice, the Δ_{24} and AUC of the number of blood monocytes increased with increasing doses of serum from B10 mice intraperitoneally injected with 1 ml latex (LaS_{24}) and 1 ml SEL ($SELS_{24}$) 24 h earlier (Figs. 5*a*, 6*a*). After the intravenous injection of LaS_{12} and $SELS_{12}$ from CBA mice, the number of monocytes in the peripheral blood of B10 mice also increased with the dose of serum (Figs. 5*a*, 6*a*). Neither LaS_{24} and $SELS_{24}$ from B10 mice nor LaS_{12} and $SELS_{12}$ from CBA mice had any monocytosis-inducing effect on CBA recipient mice over the dose range studied (Figs. 5*b*, 6*b*). Apparently, CBA mice differ from B10 mice in their ability to mount an increase of the number of monocytes in response to an injection of serum with FIM activity. The courses of the number of granulocytes and lymphocytes in the two strains did not change significantly in relation to the dose of any of the four sera under study (data not shown; for the statistical analysis of the data, see below).

Statistical Analysis of Leukocyte Response to Sera Containing FIM

For an adequate analysis of the differences in leukocyte response between B10 and CBA mice in relation to the dose of serum under study, and to find out whether other variables also contribute to the differences in response, a multiple regression analysis on all of the data shown in Figs. 5 and 6 was performed. The regression equations found for the monocyte responses expressed as Δ_{24} (Table II, equation 1) or AUC (Table II, equation 2) confirmed that the dose-dependent effect of serum is determined by the genetic background of the recipient strain.

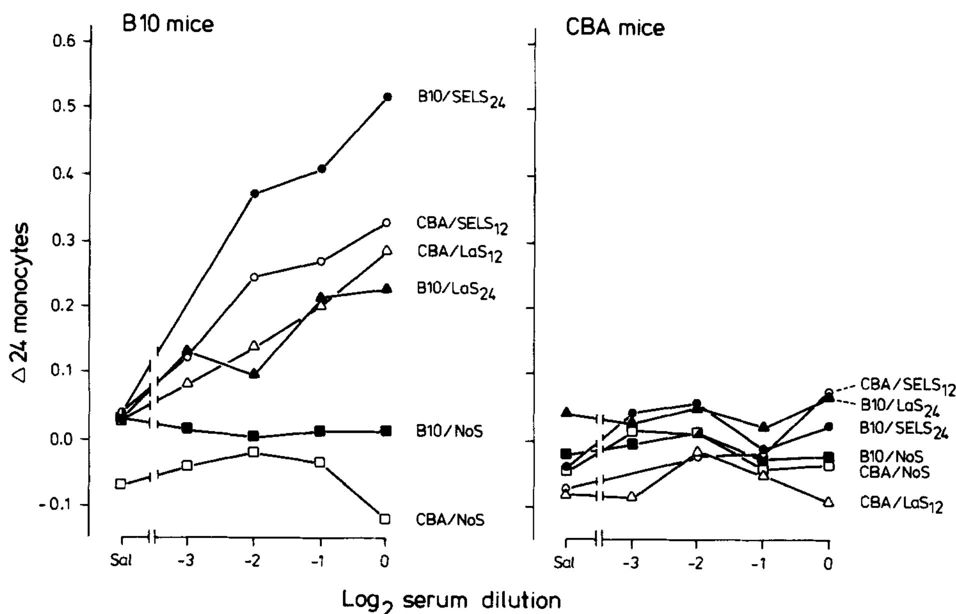

FIGURE 5. Monocyte response of C57BL/10 mice (*a*) and CBA mice (*b*) expressed as the difference between the number of blood monocytes at time zero and at 24 h after intravenous injection (Δ_{24}) of normal serum (■), LaS_{24} (▲), and $SELS_{24}$ (●) from C57BL/10 mice, and of normal serum (□), LaS_{12} (△), and $SELS_{12}$ (○) from CBA mice.

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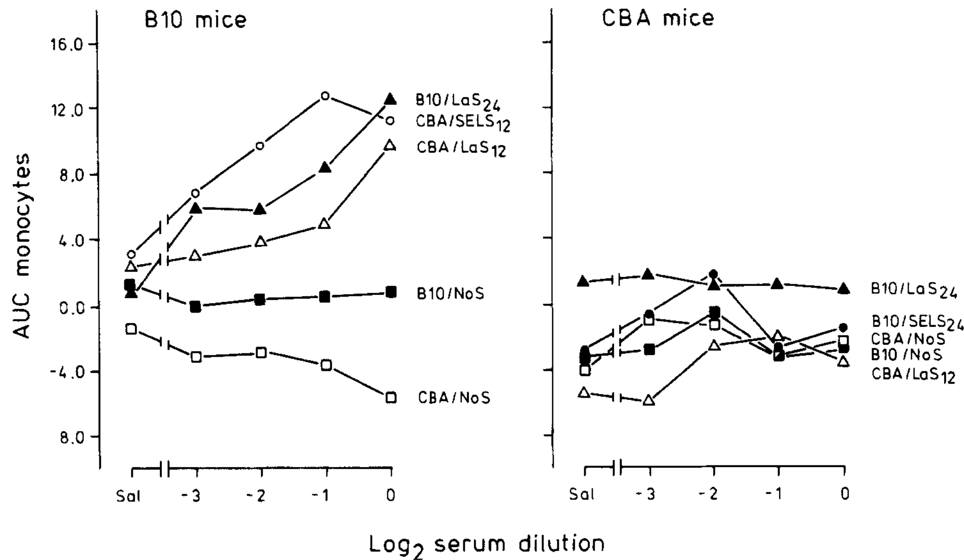

FIGURE 6. Monocyte response of C57BL/10 mice (*a*) and CBA mice (*b*) expressed as the area under the curve (AUC) of the number of blood monocytes during 72 h after intravenous injection of normal serum (■), LaS_{24}, (▲), and $SELS_{24}$ (●) from C57BL/10 mice, and of normal serum (□), LaS_{12} (△), and $SELS_{12}$ (○) from CBA mice.

TABLE II

Regression Equations of Leukocyte Responses (Δ_{24} and AUC) after Injection of Serum Containing FIM and Normal Serum into B10 and CBA Mice

Equation No.	Serum	Regression equation*	R[‡]
1	With FIM	Δ_{24} monocytes = 0.296 + 0.061 (dose × strain) + 0.321 (strain) − 0.670 ($\sqrt{N_0}$)	0.803
2		AUC monocytes = 11.23 + 2.10 (dose × strain) + 13.96 (strain) − 26.18 ($\sqrt{N_0}$)	0.843
3		Δ_{24} granulocytes = 0.931 − 0.301 (agent) − 1.087 (N_0)	0.758
4		AUC granulocytes = 33.66 − 52.55 (N_0)	0.840
5		Δ_{24} lymphocytes = 13.744 − 4.457 ($\sqrt{N_0}$)	0.699
6		AUC lymphocytes = 757.42 + 78.33 (strain) − 64.39 (agent) − 250.43 ($\sqrt{N_0}$)	0.784
7	Normal	Δ_{24} monocytes = 0.151 − 0.011 (dose) − 0.922 (N_0)	0.614
8		AUC monocytes = 6.45 − 44.98 (N_0)	0.745

* The statistical analyses for Δ_{24} values and AUC values of sera with FIM were based on 150 and 120 mice, respectively, and those for normal sera, on 80 mice. Since blood leukocyte responses of B10 and CBA mice injected with homologous SELS had been followed for only 24 h, these experiments could not be taken into account to calculate the AUC. The equations include only variables with significant correlation (at least $P < 0.05$, but usually $P < 0.00005$). Dose, the logarithm (base 2) of the serum dilution; strain, the recipient mouse strain, with the value 1 for B10 and 0 (zero) for CBA mice; agent, the stimulus used to increase the index of FIM activity, with the value 1 for latex and 0 (zero) for SEL; N_0, the number of cells × 10^{-6}/ml at time zero. Notes to equations: (Eq. 1) When B10 mice are the recipients of homologous $SELS_{24}$, the regression coefficient −0.670 must be replaced by −0.237; (Eq. 2) when B10 or CBA mice receive LaS_{12} from CBA donor mice, the terms −16.68 (N_0) and −13.86 (N_0) must be added, respectively; (Eq. 3) when CBA mice receive homologous LaS_{12} or heterologous $SELS_{24}$, the terms +0.171 ($\sqrt{N_0}$) and −0.304 ($\sqrt{N_0}$) must be added, respectively; (Eq. 4) when B10 mice receive heterologous $SELS_{12}$, the term −52.55 (N_0) must be replaced by −117.24 (N_0) and the term +63.07 ($\sqrt{N_0}$) must be added; when CBA mice receive heterologous LaS_{24}, the term −13.98 ($\sqrt{N_0}$) must be added; (Eq. 5) when CBA mice are injected with homologous LaS_{12} or heterologous LaS_{24}, the regression coefficient −4.457 must be replaced by −4.825 or −5.275, respectively; when B10 mice receive heterologous $SELS_{12}$, the term +0.177 (N_0) must be added; (Eq. 8) when B10 are injected with homologous normal serum, the regression coefficient must be replaced by −32.25.

‡ Multiple correlation coefficient.

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The CBA recipient strain had the value zero for the term strain, which means that the course of the number of blood monocytes in CBA mice was not changed significantly by the injection of serum, but depended solely on the number of monocytes at time zero. B10 mice were assigned the value 1 for the term strain, and therefore the product of dose × strain was included in the regression equation (Table II, equations 1, 2), which indicates that a dose-dependent monocytosis can be evoked in B10 mice. The intensity of this response is negatively influenced by the number of monocytes in the recipient mice at time zero, as expressed by the negative sign of the term $\sqrt{N_0}$ in these regression equations. The regression of the monocyte response on the dose of serum in B10 recipient mice is the same for all sera under study; other characteristics of the serum that could influence the slope of the regression lines, i.e., the genetic background of the donor strain and the inflammatory agent used to increase the index of FIM activity in the serum, had P-to-enter values >0.05 and were therefore not included. Taken together, this statistical analysis indicates that in sera from CBA and B10 mice, FIM is the only factor that has an effect on the monocyte response.

Similar multiple regression analyses were performed for the granulocyte and lymphocyte responses (Table II, equations 3–6). The term dose is not found in any of the regression equations, which means that sera containing FIM do not have a significant dose-dependent effect on the number of blood granulocytes and lymphocytes in either of the recipient strains.

In Vivo Responses to Normal Serum

To examine whether normal homologous or heterologous serum would influence the number of blood monocytes in B10 and CBA mice, serial twofold dilutions of serum were injected and the course of the number of monocytes was followed up to 72 h after injection. The results showed that within the dose range under study, the number of blood monocytes in both mouse strains stayed within the normal range as expressed by the Δ_{24} (Fig. 5*a* and *b*) and AUC (Fig. 6*a* and *b*) Multiple regression analysis showed that the number of blood monocytes of the recipients at time zero and the dose of serum contribute significantly ($P < 0.0005$ and $P = 0.0425$, respectively) to the number of monocytes expressed as Δ_{24} (Table II, equation 7). But compared with serum containing FIM activity, normal serum appears to reduce the number of monocytes in a dose-dependent way. Since this effect is very small and vanishes when the response is expressed as AUC (Table II, equation 8), the physiological significance of this finding is doubtful.

Injection of normal B10 or CBA serum had no significant effect on the course of the number of granulocytes or lymphocytes in B10 and CBA recipient mice (data not shown).

Discussion

The most important finding made in the present study concerns the correlation between the magnitude of the inflammatory response and the ability to respond to FIM by increased monocyte production. The inflammatory response to latex particles and SEL, expressed as the increase in the numbers of macrophages at

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the site of inflammation and monocytes in the circulation, was high in B10 mice and low in CBA mice. Both strains showed FIM in the peripheral blood after the onset of the inflammatory response. However, the intravenous injection of serum containing FIM into low responder CBA mice did not induce a response, whereas in high responder B10 mice, monocytosis was evoked. In neither of the strains did the course of granulocytes and lymphocytes change significantly after the injection of serum containing FIM, in accordance with the cell lineage-specific effect of FIM (3, 5). Since a numerical increase of monocytes after the injection of serum containing FIM reflects increased monocyte production (3, 5), we concluded that only high responder mice are able to respond to FIM by increased monocyte production.

Both ways of expressing the response to FIM, i.e., as the difference (Δ_{24}) between the number of monocytes at time zero and at 24 h after injection of a serum sample, or as the AUC of the number of monocytes during a 72-h period, are equally valid, since the correlation coefficients for both are very similar (Δ_{24}, 0.803; AUC, 0.843).

Under normal conditions, the number of peritoneal macrophages did not differ significantly between the two strains under study, but the peripheral blood of the high responder B10 mice contained slighty, but significantly, more monocytes than did the low responder CBA mice. The differences in the course of the number of macrophages in the responder strain after the administration of latex particles and SEL indicate that although the genetic constitution of the mouse strain determines the ability to react to an inflammatory stimulus, the nature of the inflammatory stimulus influences the kinetics of the inflammatory reaction. To exclude the possibility that the absence of a macrophage response in CBA mice was due to age (15), a similar study with latex as the inflammatory stimulus was done in older (i.e., 6 mo old) mice. In these mice, the number of peritoneal macrophages and the number of blood monocytes did not increase significantly (data not shown). These findings make it unlikely that the difference in magnitude of the inflammatory responses of high and low responder strains was due to the age of the low responder strain.

High and low responder mice differ with respect to the increase of monocytes and macrophages after an inflammatory stimulus. However, FIM, which is released by tissue macrophages in response to a phagocytic stimulus (17), was found in almost the same amounts in the serum of both mouse strains, but the courses of the FIM activity were not exactly synchronous. This means that a defect in the production and secretion of FIM by macrophages of the low responder strain is very unlikely. Furthermore, in both mouse strains, the number of peritoneal macrophages that had ingested latex increased with increasing doses of latex (data not shown), and analysis of covariance showed no statistically significant differences. This indicates no appreciable differences in the phagocytic activity of macrophages of CBA and B10 mice. However, others have reported functional differences, e.g., chemotactic (8) and tumoricidal activity (16), between macrophages of both mouse strains.

The innate resistance to intracellular pathogens such as *Mycobacterium bovis bacillus Calmette-Guerin*, *Salmonella typhimurium*, and *Leishmania donovani*, where macrophages are the effector cells, is regulated by genes located on chromosome

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1, possibly limited to one locus or gene complex (17, 18). The relation between this locus and the gene that controls resistance to *Listeria monocytogenes* (19) is not yet clear. The finding that, in radiation chimeras, susceptibility to *L. monocytogenes* is determined not by the characteristics of the donor mice, but by those of the recipient mice (20), is not consistent with the results of the present study. Since the mechanism underlying the difference in the response of B10 and CBA mice to injected FIM is not fully understood, the chimeras study cannot be interpreted in this respect. It seems likely, however, that the differences in FIM response involve a difference in the sensitivity of the monocyte precursor cells to FIM.

Summary

Previous studies have shown that monocyte production during an inflammatory response is controlled by the factor increasing monocytopoiesis (FIM), secreted by macrophages at the site of inflammation. The inflammatory reaction to latex particles and a saline-soluble extract of *Listeria monocytogenes* (SEL), expressed as the number of monocytes in the circulation and of macrophages at the site of inflammation, was about twice as strong in C57BL/10 mice compared with CBA mice. This raised the question as to the mechanism underlying these differences. One possibility might be that these mouse strains differ with respect to the production of FIM, but this cannot be the case because the maximum levels of FIM activity in the serum of both C57BL/10 and CBA mice given latex or SEL intraperitoneally were almost the same; however, the courses of FIM activity in the two strains after intraperitoneal latex were not exactly synchronous. Another possibility is that the sensitivity of monocyte precursor cells for FIM differs. Evidence for the latter was provided by the finding that the intravenous injection of sera with FIM activity obtained from C57BL/10 and from CBA mice into the C57BL/10 mice evoked monocytosis, whereas CBA mice did not respond to these sera. Earlier studies showed that an increase of monocytes after the injection of serum containing FIM reflects increased monocyte production. Taken together, the results of the present study demonstrate that one of the mechanisms underlying the genetic control of the inflammatory response is, rather than enhanced FIM synthesis, the ability of monocyte precursors in the bone marrow to respond to FIM by increased monocyte production.

We thank Mr. R. van Oosten for his expert biotechnical assistance and Ms. E. M. Loovens for typing the manuscript.

Received for publication 8 August 1983 and in revised form 26 October 1983.

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