The Excess Numbers of Peritoneal Macrophages in Granulocyte-Macrophage Colony-stimulating Factor Transgenic Mice Are Generated by Local Proliferation

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Summary

Mice transgenic for the hemopoietic growth factor, granulocyte-macrophage colony-stimulating factor (GM-CSF), exhibit a sustained elevation of GM-CSF levels and a 50–100-fold elevation of peritoneal macrophage cell numbers. The excess cell numbers were found to be generated in pre-adult life, with numbers remaining relatively constant thereafter. In the pre-adult period, no abnormalities were noted in the number or composition of blood, bone marrow, or spleen cells, the type or number of GM progenitor cells in the marrow or spleen, or the rate of appearance of newly formed monocytes in the peripheral blood. Peritoneal macrophages in pre-adult transgenic mice exhibited elevated mitotic activity and, after tritiated thymidine labeling, a more rapid accumulation of labeled progeny. The increase in peritoneal macrophage cell numbers appears, therefore, to be based on a GM-CSF–induced increase in local proliferative activity by peritoneal macrophages. This increased activity declined at the age of 8–10 wk, in parallel with a change in the morphology of the transgenic macrophages and an increase in binucleate and multinucleate macrophages arising by cell fusion. This change in macrophage phenotype was restricted to the transgenic mice and may therefore be a consequence of continued overstimulation by GM-CSF.

Tissue macrophages are usually regarded as having originated from blood monocytes that have seeded in the tissues, then altered their morphology and function according to their location (1). Most blood monocytes are generated by GM progenitor cells located in the bone marrow (2). The concept that tissue macrophages are derived from blood monocytes of marrow origin has been validated by an analysis of the process by which macrophages accumulate in induced peritoneal exudates (3, 4). However, the origin of resident macrophages in the peritoneal cavity of adult animals has been a more contentious issue. Kinetic analyses of resident peritoneal macrophage populations in adult parabiotic rodents have given discordant data, indicating that this population may be either partly (5) or wholly (6) self-sustaining by local mitotic activity.

The CSFs have been identified as the major regulators of granulocyte and macrophage formation in vivo (2). The intaperitoneal injection into mice of GM-CSF elicited a major rise in peritoneal macrophage numbers (7). However, only minimal changes occurred in progenitor cell levels in the marrow or spleen, and only a barely detectable rise in blood monocytes was seen. In view of the increased proliferative activity of peritoneal macrophages in these mice, the observed rise in cell numbers was likely to be due to an action of GM-CSF in stimulating local macrophage mitotic activity. Compelling evidence in support of the local origin of increased peritoneal macrophage numbers after CSF stimulation has recently been produced by studies on the administration of macrophage (M)-CSF to osteopetrotic mice (8).

Adult GM-CSF transgenic mice with constitutively elevated levels of GM-CSF in the serum and peritoneal cavity (9) exhibit, in exaggerated form, the pattern of response elicited in normal mice by the injection of GM-CSF. Adult transgenic mice were observed to have grossly elevated levels of peritoneal and pleural macrophages, yet marrow and spleen populations were near normal in numbers and composition, as were the levels of GM progenitor cells (10).

The present observations have established the time course of development of the elevated numbers of peritoneal macrophages in GM-CSF transgenic mice and have analyzed the possible origin of these excess cells during pre-adult life. The data suggest that the excess macrophages arise predominantly by enhanced local cell division in the peritoneal cavity.

Materials and Methods

Mice. Mice used were male and female members of the GM-CSF transgenic line in which insertion of the transgene is on an autosomal chromosome. The derivation of this line has been described elsewhere (10). The founder was a (C57BL × SJL)F_1 mouse and the line was maintained by crossing male transgenic mice with normal (C57BL × SJL)F_1 females. Approximately half the progeny of such matings were transgenic and these were readily

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identified by the prominent opacity of their eyes due to macrophage invasion and consequent damage to ocular tissue. The normal littermate mice were used as control mice in each experiment.

Observations. Transgenic and control littermate mice were examined between the ages of 7 and 170 d. Mice were anesthetized and white cell, hematocrit, platelet levels, and erythrocyte sedimentation rates (ESR)[1] were determined from retroorbital plexus blood. The mice were then reanesthetized and exsanguinated from the axillary vessels. The serum was separated, diluted 1:4 in 5% FCS (Hyclone Laboratories, Logan, UT) in 0.9% saline, then passed through millipore filters (Millipore Corp., Bedford, MA). A volume of 2 ml of 5% FCS/saline was injected into the peritoneal cavity and, after gentle massage, the fluid was reharvested using a soft plastic pipette. Spleens were weighed, then prepared as dispersed cell suspensions. Femur marrow cells were collected and prepared as dispersed cell suspensions in 2 ml of 5% FCS/saline. Cytocentrifuge preparations of peritoneal, spleen, and macrophage cells were stained with May-Grünwald Giemsa (BDH Chemicals, Kilsyth, Australia), coded, then typed at magnifications of 600.

Culture Assays. Progenitor cell levels in the marrow, spleen, and peritoneal populations were determined from the culture of 25,000 cells from each source in 1-ml agar medium cultures (final concentrations of 20% FCS and 0.3% agar) containing either 1,000 U of r murine GM-CSF (sp act 10^8 U/mg), or 0.1 ml of PWM-stimulated spleen conditioned medium (SCM), the latter containing 400 U each of GM-CSF and multi-CSF (2). After 7 d of incubation at 37°C in a fully humidified atmosphere of 10% CO_2 in air, colonies were counted in quadruplicate cultures, the cultures were then fixed by adding 1 ml of 2.5% glutaraldehyde. After 4 h, the cultures were floated intact onto glass slides, dried, and stained for acetylcholinesterase. These slides were then stained with Luxol-Fast-Blue and hematoxylin (BDH Chemicals). Differential colony counts were performed on coded slides at magnifications of 400 (2).

Levels of GM-CSF in transgenic and littermate sera were determined using serial twofold dilutions of each serum in microwell assay cultures containing 200 FDC-P1 cells (9). After 48 h of incubation, cell counts were performed using an inverted microscope. Observed GM-CSF levels were transformed to bone marrow U/ml by calculation from the results obtained in each assay using the half-maximal points from titrations of a standard preparation of r murine GM-CSF of known activity (100 U/ml).

Autoradiographic Analysis of GM-CSF Receptors. Purified r non-glycosylated murine GM-CSF was labeled with ^{125}I, as previously described (11). Binding to washed peritoneal cells from 10-wk-old GM-CSF transgenic or littermate control mice and subsequent autoradiographic analysis of the labeled cells were performed as described previously (12).

Tritiated Thymidine Studies. 4- and 8-wk-old transgenic and littermate mice were injected intravenously with 5 μCi/g tritiated thymidine (Amersham, Bucks, UK) in an injected volume of 0.2 ml. At intervals from 1 h to 4 d after injection, replicate smears or cytocentrifuge preparations were prepared of blood, peritoneal, or marrow cells from four separate mice on gelatin-coated slides. After fixation in methanol, these were dipped in Kodak NTB2 emulsion (Eastman Kodak, Rochester, NY) and exposed for up to 37 d. After development, the preparations were stained with May-Grünwald Giemsa, and cell labeling examined at magnifications of 600.

Statistical Analysis. Data were analyzed for statistically significant differences using the Student's t test.

[1] *Abbreviations used in this paper:* ESR, erythrocyte sedimentation rate; SCM, spleen-conditioned medium.

Results

Serum GM-CSF Levels. The transgenic state of all mice used in the study was verified by assays of GM-CSF levels in the serum. GM-CSF levels were elevated in all the transgenic mice, but at 2–3 wk of age, the degree of elevation (640 ± 380 U/ml) was slightly lower than in adult mice (1,080 ± 710 U/ml). No GM-CSF was detected in the majority of littermate sera (lower detection limit 20 U/ml), and mixing experiments showed that littermate serum did not block the ability of the assay to detect known amounts of added GM-CSF in the concentration range of 100–1,000 U/ml.

Peritoneal Cell Populations in Transgenic Versus Control Mice. Peritoneal macrophage numbers though variable, were elevated in all transgenic mice compared with littermate control mice of comparable age (Fig. 1). In some transgenic mice, cell numbers were up to 1,000-fold higher than in control mice, but in most mice the elevation was ~50-fold. Peritoneal macrophage numbers were maximal in transgenic mice at 8–10 wk of age, after which the numbers declined slightly with increasing age. As can be seen in Fig. 1, the characteristic excess of peritoneal macrophages in adult transgenic mice was achieved by a progressive logarithmic rise during the first 4 wk of life. Analysis of mice aged between 2 and 4 wk indicated that the doubling time of the macrophage population was 5.4 d in transgenic mice versus 7.8 d in control mice.

A characteristic feature of the peritoneal macrophage populations from transgenic mice was the progressive development with age of changes in the morphology of the cells. In mice aged <4-wk-old, the nucleus was of variable morphology and was surrounded by only a small rim of cytoplasm. In contrast, in adult mice the transgenic macrophages were enlarged in size, predominantly had a round nucleus, and exhibited a bulky cytoplasm (Fig. 2). Another distinctive feature of transgenic macrophages was an age-related rise in the frequency of binucleate and multinucleate cells. The frequency of binucleate macrophages commenced rising after 3 wk of age, and in most adult mice comprised 2–20% of the macrophages. The development of multinucleate macrophages with up to eight nuclei per well was slower, and these cells were not observed in transgenic mice <8 wk of age. In many transgenic mice, 5–10% of macrophages were multinucleate. GM-CSF transgenic mice of this line begin dying with a wasting disease and paresis from the age of 8 wk. However, the development of binucleate and multinucleate cells was not dependent on the development of clinical illness, since levels of both types of cell were similar in apparently healthy and moribund mice.

Blood, Marrow, and Spleen Cellularity. To determine if the increased numbers of peritoneal macrophages in transgenic mice were derived from extraperitoneal sources (blood, marrow, or spleen), an analysis was made of these extraperitoneal populations in the 2–8 wk age period during which the distinctive pattern seen in adult transgenic mice was established.

No differences in blood white cell levels, and particularly in monocyte levels, were observed between transgenic and

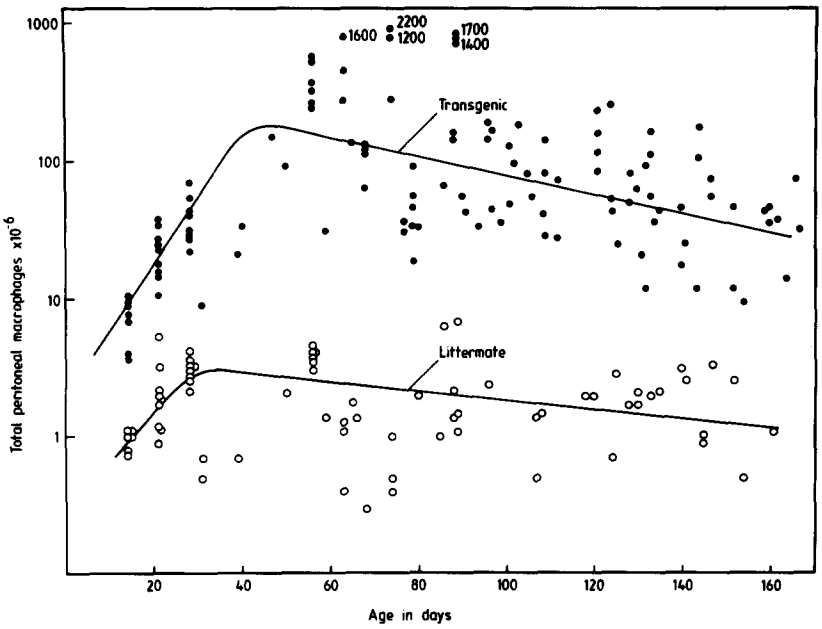

Figure 1. Change with age in the total number of macrophages in the peritoneal cavity of GM-CSF transgenic mice and littermate control mice. Each point represents data from an individual mouse.

control mice in the age range of 4–14 wk (Table 1). Hematocrit levels also were not significantly different except for low values in two 8-wk-old transgenic mice which showed bleeding in the peritoneal cavity, a feature of the disease state developing with increasing age in this transgenic line. Platelet levels in transgenic mice were slightly lower than in control mice, a trend confirmed in a more extended series of observations of apparently normal 14-wk-old transgenic mice.

In mice aged between 2- and 8-wk-old, there were no consistent differences between transgenic and control mice in total femur cell counts (Table 2), or in the composition of the marrow population. In this age range, the spleen in transgenic mice was slightly larger in weight than that in control mice, but again no major differences were observed in the size (Table 2) or cellular composition of the spleen between the two types of mice. As both types of mice progressed through this age range, the spleen switched from an erythroid to a mainly lymphoid tissue.

Progenitor Cell Levels in Transgenic Versus Littermate Mice. The frequency of GM progenitor (colony-forming) cells was determined in marrow, spleen, and peritoneal cavity cells during the period in which peritoneal cell numbers were diverging in transgenic and littermate control mice. As shown in Table 2, levels of progenitors were in fact marginally higher in the marrow and spleen of littermate mice in the 2–8-wk-old age range than in transgenic mice, but in no instance were these differences statistically significant.

Of special interest was the possibility that the transgenic peritoneal populations might contain large numbers of progenitor cells. In fact, few were detected and their frequency was lower in transgenic than in control mice (Table 2). Furthermore, the majority of the progenitor cells in the peritoneal cavity were committed to the granulocyte lineage, and would not have generated macrophage progeny. Mixing experiments using transgenic or littermate peritoneal cells with bone marrow cells from either source demonstrated no ability of peritoneal cells, in the concentrations cultured, to suppress colony formation by progenitor cells in marrow populations (data not shown).

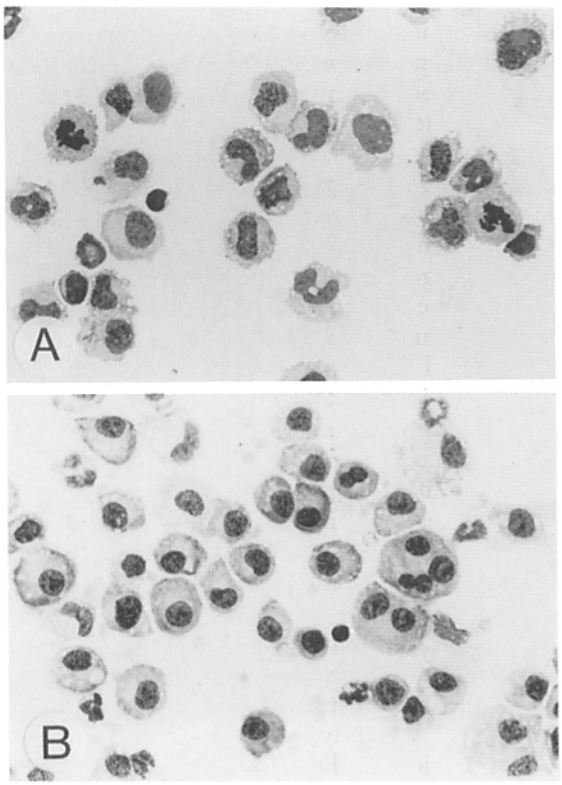

Figure 2. Morphology of peritoneal macrophages in GM-CSF transgenic mice aged (*A*) 2 wk and (*B*) 10 wk. Note the mitotic activity in the younger macrophages, the rounded nuclei of the macrophages in older mice, and the presence of binucleate and multinucleate cells.

Table 1. *Blood Cell Counts in Transgenic vs. Control Mice*

Type	Age	Total cells/μl				Platelets	Hematocrit	ESR‡
		Neutrophils	Lymphocytes	Monocytes	Eosinophils			
	wk					*× 10⁻⁶/μl*		*mm/h*
GM-CSF	4	600 ± 250*	3,750 ± 1,620	240 ± 170	40 ± 50	0.84 ± 0.21	44 ± 2	3.5 ± 1.4
Transgenic	8	510 ± 360	2,530 ± 1,700	240 ± 130	30 ± 20	0.78 ± 0.14	46 ± 2	6.3 ± 2.0
	14	880 ± 890	7,320 ± 2,800	360 ± 420	70 ± 80	0.74 ± 0.14	46 ± 3	1.7 ± 1.5
Littermate	4	580 ± 320	4,960 ± 1,090	440 ± 210	30 ± 50	0.91 ± 0.12	45 ± 1	3.1 ± 1.2
	8	280 ± 90	4,390 ± 1,610	190 ± 80	70 ± 70	1.06 ± 0.18	48 ± 1	1.6 ± 0.7
	14	670 ± 720	6,880 ± 2,300	360 ± 260	90 ± 100	0.93 ± 0.14	48 ± 1	1.6 ± 0.7

Six to eleven mice examined per group.
* Mean values ± SD.
‡ ESR, erythrocyte sedimentation rate.

The colonies developing in cultures of transgenic cells were identical in size range to those grown from control cells, and in no instance did autonomous colonies develop in unstimulated cultures. Attention was paid to the development of macrophage clusters in stimulated or unstimulated cultures of transgenic cells, in particular peritoneal cells. However, at the cell concentrations cultured, in few instances did clusters develop in cultures of peritoneal cells, and where present, these were more numerous in control cultures. A similar lack of difference in cluster frequency was noted in cultures of marrow or spleen cells.

Morphological typing of the colonies developing in transgenic cultures indicated no unusual relative frequency of granulocyte, granulocyte macrophage, macrophage, or eosinophil colonies compared with cultures of cells from control mice of the same age.

Local Peritoneal Macrophage Mitotic Activity. The failure to find any significant differences between the blood, marrow, or spleen populations between transgenic and control mice directed attention to possible differences in the local behavior of peritoneal macrophages as the basis for the observed differences in cellularity.

No mitotic activity was observed in the lymphocytes, eosinophils, neutrophils, or mast cells in the peritoneal cavity of either transgenic or control mice. However, mitotic figures were clearly evident in peritoneal macrophages from 2–4-wk-old mice. In 24 mice of each type, aged 2–4 wk, the frequency of mitosis per 10^3 macrophages was significantly

Table 2. *GM Progenitor Cell Frequency in the Marrow and Spleen of Transgenic vs. Littermate Mice*

Type	Age	No. of mice	Total marrow cells	Spleen weight	Progenitor cells/2.5 × 10^4 cells		
					Marrow	Spleen	Peritoneal cavity
	wk		*× 10⁻⁶*	*mg*			
GM-CSF	2	7	14.2 ± 5.9	68 ± 17	29 ± 9	12 ± 5	0
Transgenic	3	9	19.3 ± 5.1	91 ± 50	43 ± 15	11 ± 10	0.8 ± 1.4
	4	8	23.5 ± 4.5	128 ± 37	46 ± 12	12 ± 7	0.1 ± 0.2
	8	6	35.4 ± 3.2	159 ± 23	46 ± 9	2 ± 2	0.4 ± 0.5
Littermate	2	7	15.3 ± 4.8	49 ± 4	38 ± 13	17 ± 7	0.9 ± 0.9
	3	9	20.8 ± 8.2	64 ± 13	50 ± 7	13 ± 5	0.9 ± 1.1
	4	8	27.5 ± 6.4	91 ± 25	50 ± 13	9 ± 8	0.3 ± 0.4
	8	6	35.6 ± 5.2	95 ± 31	59 ± 10	6 ± 6	0.2 ± 0.3

Marrow cell counts represent total cells from one femur per mouse. Mean values ± SD. All cultures contained 25,000 cells and were stimulated by 0.1 ml PWM-stimulated spleen conditioned medium.

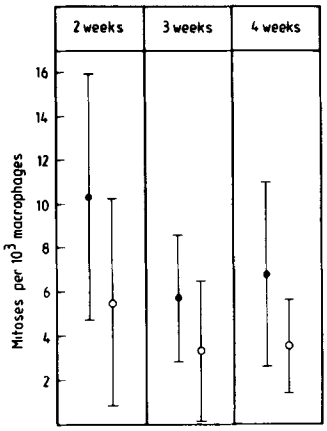

Figure 3. Frequency of mitoses per thousand peritoneal macrophages in transgenic (●) and littermate control mice (○). Mean values ± SD of groups of 8–10 mice at each age.

higher in transgenic mice (7.1 ± 4.7) than in control mice (4.0 ± 3.4) ($t = 2.95$, $p < 0.01$). As shown in Fig. 3, the frequency of mitoses fell in mice of both types with increasing age, but at each time point the frequency was higher in transgenic than in control mice.

The likely differences in peritoneal macrophage proliferative activity were examined in more detail by autoradiography of cells from mice injected with tritiated thymidine. A sequential analysis was made of mice of two ages: (*a*) 4 wk of age, when an active increase was occurring in peritoneal macrophage numbers, and (*b*) 8 wk of age, when total peritoneal cell numbers had achieved their maximum levels.

As shown in Fig. 4, in 4-wk-old mice, the percentage of macrophages in S phase and labeled at 1 h after thymidine injection in transgenic mice (9 ± 2%) was almost double the percentage in littermate mice (5 ± 0.5%). After initial labeling, the percentage of labeled cells rose progressively during the next 72 h, reaching 45 ± 5% in transgenic mice, but only 18 ± 2% in littermate mice. At 72 h, levels of labeled cells appeared to be approaching a plateau in mice of both types. The number of available animals did not permit a more complete analysis of the mitotic cell cycle, but the fall in mean grain counts with time indicated that cell cycle times of labeled cells were <24 h, much shorter than the observed doubling times for the populations. This indicates that two subsets of cells were probably present in the peritoneal macrophage population — a smaller mitotically active subset and a larger, quiescent population. The data suggested that the major difference between the two types of mice was the higher proportion of transgenic macrophages in the mitotically active pool. It was evident from the grain counts of labeled cells at 48 and 72 h that many intensely labeled cells were present in mice of both types at these late time points, indicating that some progeny of the cells that had been in S phase at the time of initial labeling had not undergone further cell divisions, but had left the mitotic pool. This would account for the failure of the percentage of labeled cells to rise uniformly with time, since an increasing number of labeled cells were no longer exhibiting mitotic activity.

The labeling pattern in 8-wk-old mice was sharply different

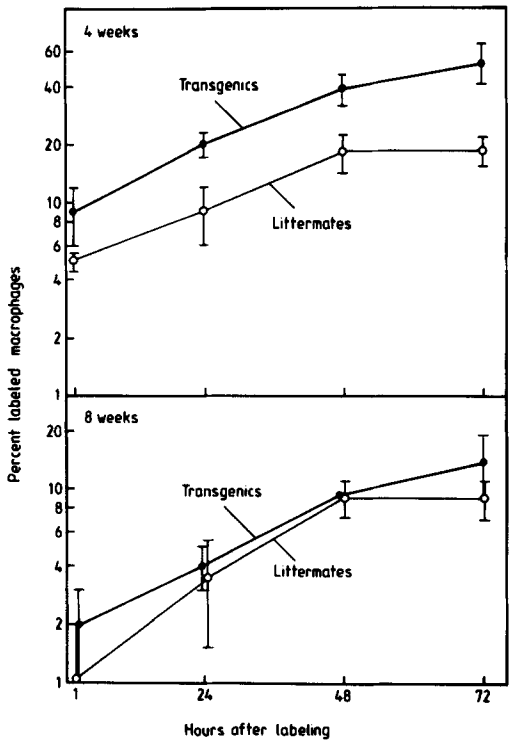

Figure 4. Frequency of labeled macrophages in transgenic and littermate control mice at intervals after pulse labeling by the intravenous injection of tritiated thymidine. Mean values ± SD of four separate mice at each time point.

from that in 4-wk-old mice. Whereas the percentage of labeled macrophages was again higher in transgenic than in littermate mice, only 2 ± 1% of transgenic macrophages were in S phase at the time of labeling, and by 72 h only 16 ± 4% of cells were labeled, a rate of increase in percent labeled cells not significantly different from that observed in littermate control mice.

These data suggest that a marked reduction had occurred in the relative size of the active pool of macrophages by the age of 8 wk, with little difference in the behavior of this population between the two types of mice at a time when total cells numbers were being maintained at a stable level.

One possible basis for the decline in mitotic activity of adult transgenic macrophages might have been the failure of these cells to continue to express membrane receptors for GM-CSF. However, as shown in Fig. 5, an autoradiographic analysis of macrophages from 10-wk-old transgenic mice after labeling in vitro with ^{125}I–GM-CSF showed not only the continuing presence of receptors on all transgenic macrophages, but also that receptor numbers were higher (grain counts per cell) than on macrophages from the peritoneal cavity of littermate control mice of the same age.

Analysis of the tritiated thymidine labeling pattern of binucleate and multinucleate transgenic macrophages indicated, as has been shown in other systems where multinucleate macrophages develop (13, 14), that these cells arose by fusion rather than by endomitosis. The cell shown in Fig. 6 can have

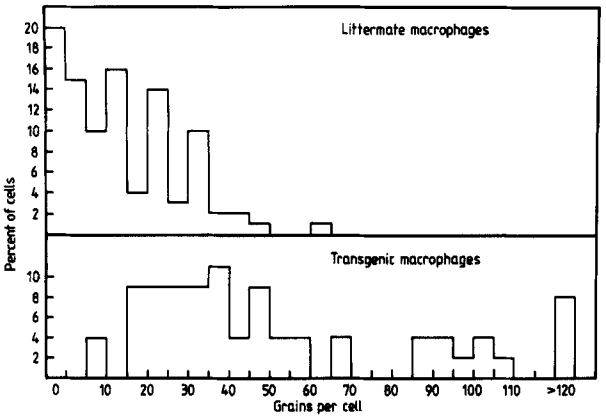

Figure 5. Autoradiographic analysis of the binding of ^{125}I-GM-CSF to peritoneal macrophages from GM-CSF transgenic and littermate control mice aged 10 wk. Grains/cell reflects the frequency of GM-CSF receptors on individual cells.

arisen only by fusion of an unlabeled cell with at least two other labeled cells. This conclusion is supported by the failure to find labeling of any nucleus in binucleate or multinucleate cells 1 h after labeling, indicating that none of these cells were in S phase. Furthermore, an analysis of binucleate cells from mice 72 h after labeling showed a combination of a labeled and an unlabeled nucleus in 15 of 177 binucleate cells, and labeling of both nuclei in 2 of 177 cells. In 50 multinucleate cells, labeling of one or two nuclei was observed in nine of the cells. The frequency of labeled binucleate and multinucleate cells was lower at 72 h than that of transgenic macrophages with a single nucleus. Nevertheless, the data suggested that cell fusion did not involve exclusively aged cells as the presence of a labeled nucleus in such cells indicated, at least for these cells, that one of the fusion partners recently had been in active cell cycle.

The accumulation with time of labeled monocytes in the bone marrow and blood of transgenic animals was similar to that in control mice. Since blood monocyte levels were similar in both types of mouse, these data exclude the possibility that monocytes were transiting from the marrow to the blood of transgenic mice more rapidly than in control mice or that, in transgenic mice, greatly increased numbers of monocytes could have entered the peritoneal cavity from the blood. Furthermore, at 1 h after the administration of tritiated thymidine, when some peritoneal cells were labeled, blood monocytes were unlabeled. At 24 h, the labeled peritoneal macrophages did not resemble the morphology of monocytes, again indicating that the transgenic mice were not exhibiting a massive acceleration of entry of labeled cells from the blood into the peritoneal cavity.

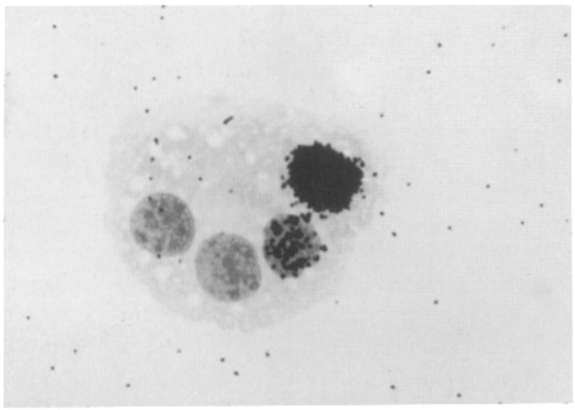

Figure 6. A single multinucleate macrophage from a GM-CSF transgenic mouse 72 h after labeling with tritiated thymidine. The presence of two nuclei with differing labeling intensities indicates that the cell arose by fusion.

Discussion

The present observations on GM-CSF transgenic mice have sought to establish the origin of the massively increased numbers of peritoneal and pleural macrophages in adult mice of this type. The data indicated that, in adult mice, peritoneal macrophage numbers are relatively constant and, rather than increasing with age, actually decrease slightly. The abnormally high levels of macrophages in transgenic mice were achieved in the first 8 wk of life, indicating that the cellular basis for the difference needs to be established by studies on mice in the pre-adult period.

In principle, the massively increased numbers of peritoneal macrophages in transgenic mice either could have come from an increased production of monocytes in the marrow and spleen with a subsequent increased delivery of monocytes via the blood to the peritoneal cavity, or could have arisen from increased local macrophage proliferative activity in the peritoneal cavity itself.

The pre-adult period was characterized as a time when circulating GM-CSF levels were already markedly elevated. No abnormalities were detected during the pre-adult period in bone marrow or spleen cell numbers, levels, or types of progenitor cells in these tissues, blood monocyte levels, or rates of appearance of newly-formed monocytes in the peripheral blood. The rise in peritoneal cellularity therefore appears to be achieved without any detectable perturbation of cell populations generating monocytes that might have migrated into the cavity. In vitro analysis of the peritoneal populations failed to reveal an elevated frequency of progenitor (colony-forming) cells in the local population but, because of the differing total cell numbers, the total number of progenitor cells was increased, possibly 20–50-fold. As most of these progenitors were committed to the granulocyte lineage, they would not have contributed to local macrophage production. However, some increase in absolute numbers of macrophage progenitors can be concluded to have been present and to have contributed to the observed increase in local macrophage proliferative activity, although most of the dividing cells were likely to have been more mature cells.

GM-CSF appears to be a free exchange between the serum and the peritoneal cavity with GM-CSF concentrations being similar in both locations (9). The situation documented in transgenic mice therefore mimics, in exaggerated form, the

response of mice to the intraperitoneal injection of GM-CSF in which little change is observed in blood, marrow, or spleen populations in contrast to the marked rise in the number and local mitotic activity of peritoneal macrophages (7).

The peritoneal macrophages in pre-adult transgenic mice exhibited elevated mitotic activity sufficient to account for the shortened doubling time of the population observed during this period, given the likely cell cycle times for dividing murine macrophages of 12–20 h, (15, 16). The labeling studies using tritiated thymidine indicated that not all transgenic peritoneal cells were in active cell cycle. Instead, the mitotic activity was restricted to a subset of these cells and many of the progeny subsequently entered a non-cycling pool. The major difference between the transgenic and control mice was the larger size of the cycling subset in transgenic mice. In young transgenic mice, possibly more than 20% of the macrophages were in the cycling pool, versus about 10% in the control mice. The observations made were not able to establish whether the length of the cell cycle in these dividing macrophages also differed between transgenic and normal mice and whether some noncycling transgenic macrophages were able to reenter the dividing pool.

The origin, therefore, of the grossly increased peritoneal, and presumably pleural, populations of macrophages in GM-CSF transgenic mice appears to be a GM-CSF–induced increase in the proportion of the population able to exhibit proliferation—a response similar to that observed after the local injection of GM-CSF in normal mice (7). A dramatic example of the ability of local CSF to induce major rises in the local peritoneal macrophage populations without inducing comparable changes in marrow, spleen, or blood monocytes populations, has been documented recently by studies on the responses of osteopetrotic (op/op) mice to the administration of M-CSF (8).

Since GM-CSF levels remain grossly elevated in the transgenic mice throughout life, it is interesting that peritoneal cell numbers did not rise progressively beyond 8–10 wk of life. For some reason, the nature of the peritoneal macrophage population changes qualitatively after 8–10-wk-old in GM-CSF transgenic mice. After this time, the morphology of the cells is radically altered from that in younger mice as shown in Fig. 2, and there is a progressive increase in the proportion of binucleate and multinucleate cells. Since these changes were not seen in normal mice and the peritoneal macrophages in normal adult mice remain responsive to proliferative stimulation by injected GM-CSF (7), the changes must be presumed to be long-term consequences of overstimulation by GM-CSF. The macrophages in older transgenic mice do, however, continue to express receptors for GM-CSF and to exhibit heightened functional activity as assessed by superoxide and IL-1 production (17, 9), but they are no longer capable of exhibiting increased mitotic activity.

Does this suggest that, after sustained proliferative stimulation by CSFs, a responding population eventually becomes refractory to further proliferative stimulation? This seems unlikely since patients with cyclic neutropenia have remained responsive to stimulation by injected G-CSF for several years (18). This suggests, therefore, that peritoneal macrophage populations, as quasi-end-cells, exhibit an atypical response to continued proliferative stimulation, at least by GM-CSF, and are induced to alter their morphology and to remain capable of exhibiting heightened functional responses, but not increased cell division. That these changes are peculiar to cells in the peritoneal and pleural cavities is indicated by the failure to observe multinucleate cell formation in the marrow or spleen populations.

These observations have raised some unanswered questions regarding the nature of long-term responses to CSF stimulation, and the study has provided strong evidence that resident peritoneal macrophages in GM-CSF transgenic mice behave as an essentially independent cell population, expanding abnormally, then maintaining itself largely by local cellular proliferation.

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