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Impaired Chemotactic Responsiveness of Macrophages from Gnotobiotic Rats

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Peptone-induced macrophages obtained from gnotobiotic (GB) rats responded poorly to chemotactic stimuli that have a powerful, attractive influence upon the cells of conventional donors. Monocyte recruitment from the circulation into peptone-induced exudates also was impaired in GB subjects. Although relatively more resident cells are present in exudates borne by GB donors, their number cannot in itself account for the sluggish response of peptone-induced cells from GB rats. Neutrophil accumulation in the inflamed peritoneal cavities and their responsiveness in vitro were similar in GB and conventional rats. The levels of serum-derived chemotactic factors were similar in such animals. Furthermore, germ-free rats exhibited no obvious defects in their capacity to generate lymphocyte-dependent monocyte chemotactic activity in situ upon specific stimulation with *Listeria monocytogenes*. It is suggested that the diminished chemotactic responsiveness of exudate macrophages is related in some way to the level of cell activation. This state of affairs might account for the impairment of delayed-type hypersensitivity in GB animals and their inability to resist intracellular bacterial infections.

Germ-free (GF) animals have a low level of resistance to the tubercle bacillus and to other infectious agents that provoke a vigorous cell-mediated immune response in conventional (CONV) subjects (31). It also has been reported that GF guinea pigs (14, 15) and GF mice (32, 33) either fail to express delayed-type hypersensitivity (DTH) or show only feeble reactions to antigens that elicit florid responses in CONV animals. Unresponsiveness cannot be ascribed to a general failure of T-cell function, because GF animals can produce circulating antibodies to thymus-dependent antigens (5, 29), and their capacity to reject allografts is unimpaired (25). Therefore, the question arises whether the immune deficiency in GF subjects is related to a defect in macrophages. It is in keeping with this notion that the macrophages of GF animals are often less active metabolically and lack the microbicidal capability of their CONV counterparts (7). Furthermore, macrophages are indispensable participants with activated T cells in the expression of DTH (16) and cellular resistance to infection (17, 21, 35), the very responses that are most severely compromised in the GF animal.

Results of the current study substantiate this view insofar as they demonstrate that exudate macrophages from gnotobiotic (GB) and GF rats respond poorly to chemotactic stimuli that exert strong attraction on the macrophages of CONV donors. By comparison, neutrophil chemotaxis is not obviously impaired, and GB and CONV rats do not differ in their capacity to generate either serum-derived chemotactic factors or antigen-induced monocyte chemotactic activity (MCA). The observed dysfunction of macrophages from GB rats suggests that the failure of GF animals to express DTH or defend themselves against intracellular bacterial infections is related to the inability of the host to marshal monocyte-derived macrophages into lesions.

MATERIALS AND METHODS

Animals. GB female Sprague-Dawley rats weighing 150 to 225 g were purchased from Charles River Co., Bar Harbor, Maine. Some, but not all, of these animals harbored in their intestine an unidentified gram-positive anaerobic bacterium. The animals were free from other bacteria, mycoplasma, fungi, parasites, and 12 common pathogenic viruses of rodents. GB rats were shipped in sterile containers to the Trudeau Institute, Saranac Lake, N. Y., where they were transferred into isolators and allowed to acclimatize for 1 to 2 weeks. CONV Sprague-Dawley (Charles River) females, weight matched with the GB rats, were used at the Baker Institute 1 week after arrival. Male (Lewis × DA) F_1 hybrid rats and GF Fischer rats were bred at the Trudeau Institute. These were used as a source of indicator macrophages and of lymphocyte-dependent MCA.

Confirmation of GB status. The macrobial status of GF and GB animals was kindly determined by P. B. Carter. Intestinal contents were incubated at 37°C in thioglycolate broth and plated on blood agar under both aerobic and anaerobic conditions. In addition, wet mounts and Gram-stained fecal smears were inspected microscopically. Failure to detect microorganisms in the smears and cultures after 120 h was taken as evidence that the intestinal lumen was "sterile." Uteri and lungs were examined for mycoplasms by culture in mycoplasma broth and by indirect immunofluorescence with antisera to *Mycoplasma pulmonis*.

Cells. A 7- to 8-ml portion of a 10% (wt/vol) solution of autoclaved myosate peptone (Baltimore Biological Laboratory, Cockeysville, Md.) in saline was injected intraperitoneally (i.p.). Exudates induced in this manner were harvested after 10 to 12 h to obtain a cell population rich in neutrophils. Similarly induced exudates were harvested at 72 h to obtain a population enriched in macrophages. In each case, the peritoneal exudate cells (PEC) were collected in 20 to 30 ml of cold Hanks balanced salt solution containing 2 U of preservative-free heparin per ml. The cells were centrifuged from the medium at 200 × *g* for 10 min and washed twice with Hanks balanced salt solution.

Chemotactic stimuli. Normal rat serum that had been heated for 30 min at 56°C was used as an attractant for both neutrophils and macrophages. Another attractant for macrophages was prepared by incubating 400 μg of alcohol-killed lyophilized *Listeria monocytogenes* in 50 μl of saline to which 1 ml of normal rat serum was added (8). The mixture was incubated for 30 min at 37°C and again for 30 min at 56°C. Thereafter, the mixture was centrifuged for 6 min at 3,000 × *g*, and the supernatant was removed, membrane-filtered (Millipore Corp.), and diluted to the desired concentration (usually 10%). The pH of the sera was adjusted to 7.1 to 7.2 immediately before use in experiments. In some experiments, preformed immune complexes rather than *L. monocytogenes* were used for the activation of serum (11). These were prepared with human serum albumin and a rabbit antibody to human serum albumin.

MCA was generated in situ in 6-day *Listeria*-immune rats by injecting such animals i.p. with 50 μg of killed *L. monocytogenes* (11). Exudates induced in this way were harvested after 24 h with 3 ml of Hanks balanced salt solution. PEC were removed by centrifugation. The supernatant was then filtered in a membrane (Millipore Corp.) and stored at −70°C.

Measurement of chemotaxis. Chemotaxis was measured in vitro by using a modified Boyden chamber technique (8). For the measurement of macrophage chemotaxis, the chambers were fitted with 8-μm filters (Sartorius Membranfilter no. 11301, Göttingen, West Germany). Portions of 2 × 10^6 macrophages were dispensed in 0.5 ml into the upper compartment of each of two to three chambers that contained a chemotactic substance in the lower compartment. The chambers were incubated at 37°C for 5 h before the filters were removed, fixed, and stained. Macrophages that had migrated to the lowermost filter level were counted in four representative fields. Each field equaled 1/560 of the filter surface. The results are expressed as mean number of macrophages per field ± standard deviation.

Neutrophil chemotaxis was measured by a similar technique (10) by using chambers that were equipped with 3-μm filters (Sartorius Membranfilter no. 11302). Here chambers were charged with 1.25 × 10^6 neutrophils, and the incubation time was reduced to 4 h.

Experimental procedure. Studies at the Trudeau Institute and the Baker Institute were performed under strictly comparable conditions with identical reagents.

Student's *t* test (26) was used for statistical analysis of Fig. 1, 2, and 3 and Tables 1 and 2. Unless otherwise indicated, a level of significance higher than 0.01 was regarded as significant.

RESULTS

Accumulation of macrophages in peptone-induced exudates. Groups of 10 to 12 GB and CONV rats were stimulated i.p. with 7 ml of peptone. Exudates were collected from these animals after 3 days. An additional 10 CONV rats and 5 GB subjects were sacrificed to determine the number of macrophages in the unstimulated peritoneal cavity.

Figure 1 shows that the numbers of macrophages in exudates borne by stimulated rats exceeded the number present in the unstimulated peritoneal cavities of their respective con-

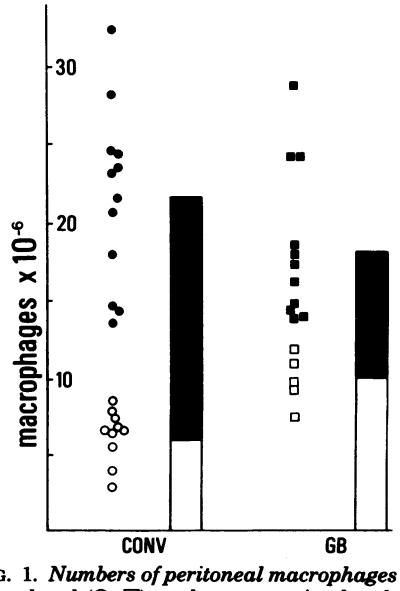

FIG. 1. *Numbers of peritoneal macrophages in the unstimulated* (○, □) *and peptone-stimulated* (●, ■) *peritoneal cavities of CONV and GB Sprague-Dawley rats. Bars represent means of groups.* (□) *Unstimulated cavity;* (▤) *net influx following peptone stimulation (67% for CONV, 43% for GF rats). After peptone stimulation, significantly more macrophages were recruited from the circulation into the cavity of CONV rats.*

trols. These differences are ascribed to an influx of monocyte-derived macrophages in response to inflammation. The small differences in numbers of cells recovered from GB as opposed to CONV donors were not significant ($P > 0.05$). But the peritoneal cavities of unstimulated GB rats contained significantly more peritoneal macrophages than their CONV counterparts ($P < 0.01$). In reality, therefore, CONV animals marshaled into exudates more monocyte-derived macrophages than did weight-matched GB subjects ($P < 0.05$).

Chemotactic response of macrophages. Macrophages from peptone-induced exudates were tested in vitro for their response to chemotactic stimuli. In one experiment, PEC obtained from GB and CONV donors were pooled within groups and added in equal numbers (4×10^6 macrophages/ml) to chemotaxis chambers. The latter contained either heated rat serum or immune-complex-activated serum in the lower compartment. Resident macrophages from the unstimulated cavities of CONV donors also were tested.

The results are summarized in Table 1 where exudate macrophages were far more responsive to both attractants than were the cells from unstimulated donors. But the exudate macrophages of GB rats responded poorly when compared with similarly elicited macrophages from flora-bearing rats.

Similar results were obtained in another experiment in which macrophages from individual donors were assayed for responsiveness to the same chemoattractants (Fig. 2). Variation was observed from animal to animal. It is evident nonetheless that exudate macrophages from CONV donors were more responsive than the cells of their GB counterparts ($P < 0.01$).

In this experiment, resident macrophages of CONV subjects failed to respond to heated serum and were only feebly attracted toward complement-activated serum. The above finding raises the question whether the sluggish behavior of PEC from GB donors is related to the presence in the exudate of a relatively large portion of resident macrophages. To test this possibility, cell mixtures were prepared containing different proportions of resident (unresponsive) cells and elicited (responsive) cells from CONV donors. All chambers were charged with a total of 2×10^6 macrophages. An additional set was loaded with 10^6 resident and 2×10^6 elicited test cells to determine the effect of "cell crowding."

Increasing the number of PEC in the cell mixture enhanced the migration of macrophages toward heated serum (Fig. 3). The response was directly proportional to the number of responsive cells in the chamber, but only when small numbers of elicited cells or a weak attractant (50% serum) was used. Addition to the chambers of a larger number of cells or a more potent attractant (neat serum) gave a proportionately smaller increase in response. The above finding can be explained, at least in part, by a crowding effect. Thus, the addition of 10^6 "unresponsive" cells significantly impaired the migration of 2×10^6 "responsive" cells through the membrane (not shown).

The dose-response relationship of Fig. 3 can be used to estimate the response that might be expected of cell populations containing resident macrophages in proportions similar to that observed in exudates obtained from GB rats (57%; Fig. 1). It is evident that the decreased responsiveness of macrophages from GB donors cannot be explained solely by the larger number of resident cells in the test cell population (Fig. 2; Table 1).

Neutrophil response in vivo and in vitro. The results of the foregoing experiments point to an intrinsic defect in the capacity of GB macrophages to respond in vitro to chemotactic stimuli. To determine whether a similar defect exists in neutrophils, GB and CONV rats were stimulated i.p. with peptone. PEC obtained from these animals after 10 to 12 h contained many neutrophils, regardless of the microbial status of the donor. Indeed, neutrophil numbers were

TABLE 1. *Responsiveness of various macrophage pools to serum-derived chemotactic factors*

Chemoattractant		Chemotactic activity[a]		
Agent	Concn (%)	Elicited macrophages from:		Resident macrophages from CONV rats
		CONV rats	GB rats	
Heated rat serum[b]	100	319.0 ± 29.1	112.3 ± 13.2	12.2 ± 3.8
Immune complex-activated rat serum[c]	10	204.7 ± 39.8	99.5 ± 26.4	24.5 ± 7.4

[a] Macrophages per field (mean ± standard deviation).
[b] Heated for 30 min at 56°C.
[c] See Materials and Methods for details.

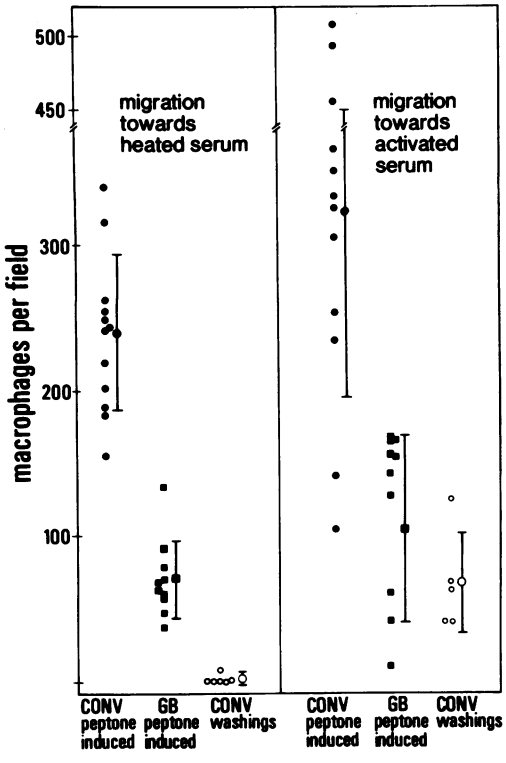

FIG. 2. *Chemotactic responsiveness of various PEC populations towards serum-derived attractants. Peptone-induced macrophages from CONV rats show much more vigorous response than those from GB rats. Resident cells from CONV rats were either weakly responsive or unresponsive.*

even higher in GB exudates [(126 ± 46) × 10^6] than in exudates borne by CONV subjects [(57 ± 25) × 10^6].

Cells from the two groups of donors were pooled within groups and tested for their chemotactic responsiveness toward "activated" rat serum. Table 2 shows that neutrophils of GB rats were not obviously defective in their capacity to respond to the factors concerned. But a subtle difference in responsiveness cannot be discounted categorically. Thus, GB neutrophils responded optimally to relatively lower concentrations of chemoattractant than their counterparts from CONV donors (indexes, Table 2).

Humoral chemotactic activity. A further comparison of GB and CONV rats was made by measuring the ability of their sera to attract peritoneal macrophages from CONV donors. Both fresh-frozen and heat-inactivated sera were tested. Over a broad concentration range, similar activities were detected for sera withdrawn from GB and CONV PEC donors (Fig. 4). The same experiment and other data (not shown) revealed that peptone injection 3 days before collection of the sera did not significantly alter their chemotactic properties.

The factor(s) responsible for chemotactic activity of fresh-frozen and heated serum has not been identified; however, there are reasons for thinking that it is not a product of the complement system (4, 6, 9). It was of interest, therefore, to compare the levels of complement-derived activity in the serum of GF and CONV rats. To this end, portions of fresh sera obtained from the same donor rats were activated in vitro

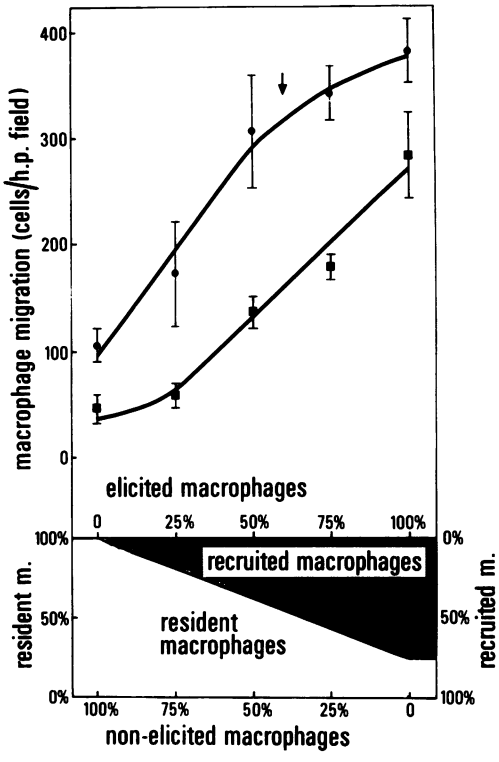

FIG. 3. *Chemotactic response towards 100% (●) and 50% (■) heated homologous serum of various mixtures of elicited and nonelicited PEC (mean of 12 ± standard deviation). All chambers contained 2 × 10^6 macrophages. The proportion of macrophages recruited from the circulation was calculated as follows: (mean elicited macrophage/rat − mean resident macrophage/rat in unstimulated control group):mean elicited macrophage/rat. The assumption was made that 3-day elicited exudates contained the same number of resident cells as before stimulation. Mixtures of elicited and nonelicited macrophages contained proportionally less recruited macrophages than pools of elicited cells alone. Arrow indicates the expected response of a PEC mixture similar to that of peptone-induced exudates in GB rats, e.g., 43% of elicited and 57% of resident cells (see Fig. 1).*

TABLE 2. *Chemotactic response of pooled exudate neutrophils to various chemotactic agents*

Chemotactic prepn	Concn (%)	Chemotactic activity[a]		
		CONV	GF rats	Ratio (GF:CONV)[b]
Immune complex-activated heated rat serum[c]	20	304.8 ± 42.6	199.3 ± 55.2[d]	0.654
	10	333.3 ± 41.6	230.0 ± 35.0[d]	0.690
	5	307.3 ± 42.8	282.0 ± 30.9	0.918
Listeria-activated heated rat serum[e]	20	318.5 ± 40.1	253.0 ± 45.1[d]	0.794
	10	289.8 ± 51.9	289.0 ± 29.3	0.997
	5	187.3 ± 22.2	254.0 ± 18.3	1.356
Heated rat serum[f]	20	57.9 ± 12.7	ND[g]	
	10	103.6 ± 26.7	115.9 ± 35.3	1.119
	5	119.0 ± 26.5	ND	
M 199 (control)		9.5 ± 4.7	14.4 ± 2.5	

[a] Neutrophils per field (mean ± standard deviation).
[b] Geometric mean of all ratios = 0.905.
[c] See Materials and Methods for details.
[d] Significantly lower than in conventional rats ($P < 0.01$).
[e] See Materials and Methods for details.
[f] Serum was heated for 30 min at 56°C, the pH was then adjusted, and the dilutions were made with Hanks balanced salt solution.
[g] ND, Not done.

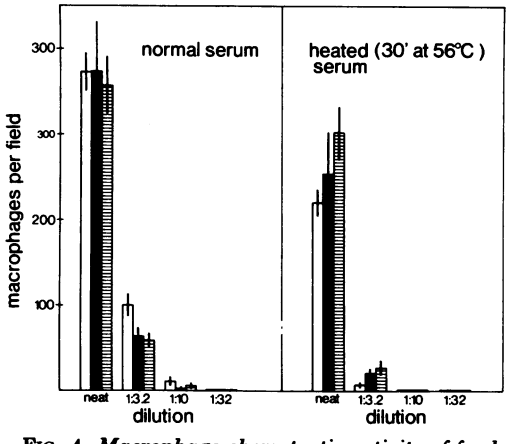

FIG. 4. *Macrophage chemotactic activity of fresh-frozen and heated rat serum from various donors. (□) Untreated conventional rats; (■) conventional rats 3 days after i.p. injection of peptone; (▤) gnotobiotic rats 3 days after peptone injection. Various dilutions were tested. Dose response pattern is similar for all sera.*

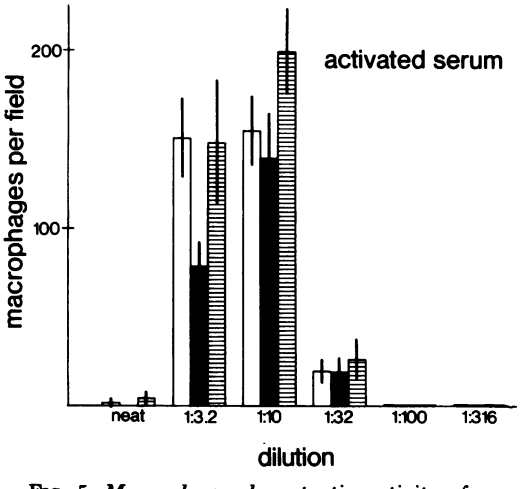

FIG. 5. *Macrophage chemotactic activity of various dilutions of L. monocytogenes-activated rat sera. Various dilutions were tested. See legend to Fig. 4 for symbol explanation. Dose response pattern is similar for all sera.*

with *L. monocytogenes*, thereby generating complement-derived chemotactic activity.

Figure 5 indicates that the activated sera of GB and CONV donors exhibited approximately the same level of chemotactic activity.

Generation of lymphocyte-dependent MCA in GB and CONV rats. Injection i.p. of *L. monocytogenes* into specifically immunized rats stimulates the formation of lymphocyte-dependent MCA (11). Since the above factor has been implicated in the expression of DTH (11), and since DTH reactions are impaired in GF subjects (14, 15, 32, 33), it was of interest to compare the generation of MCA in GF and CONV subjects. To this end, six GF Fischer and a group of CONV Lewis × DA F_1 hybrid rats were immunized in the forepaws with living *L. monocytogenes*. Other animals (both GF and CONV) were reserved as nonimmunized controls. After 6 days, half the immunized rats in

each group and all the nonimmunized subjects were injected i.p. with 50 μg of killed *Listeria*. All were euthanized 24 h after stimulation. Peritoneal fluids from individual animals (3 ml) were pooled within groups and assayed for chemotactic activity, using macrophages from either GF or CONV donors as indicator cells.

The data in Table 3 conform with those obtained in earlier experiments insofar as the macrophages from CONV rats migrated far more vigorously toward both activated serum and peritoneal fluid than did the cells of GB donors. Whereas the macrophages of GB donors respond poorly or not at all to MCA in vitro, it is evident that the intact animal was able to generate MCA, since exudates induced in specifically sensitized GF rats had a powerful attractive influence upon PEC from flora-bearing subjects. The level of MCA in exudate supernatants from GF Fischer strain donors was lower than that detected in similarly induced exudates borne by CONV LDA rats. However, the observed difference may be more apparent than real, because it had been shown previously (11) that CONV Fischer rats give relatively feeble MCA responses when stimulated by *L. monocytogenes*. It would seem, therefore, that GB rats are able to generate lymphocyte-dependent MCA although their exudate macrophages respond poorly to it.

DISCUSSION

The present study demonstrates that elicited macrophages from the peritoneal cavity of GB rats respond poorly in vitro to chemotactic stimuli that have a powerful attractive influence upon the macrophages of CONV subjects (Table 1; Fig. 2). It also was observed that GB rats are less efficient than their CONV counterparts in their capacity to marshal macrophages into peptone-induced exudates (Fig. 1). Since the unstimulated peritoneal cavity of GB rats contains a relatively large number of resident macrophages, and since resident macrophages respond poorly to chemotactic stimuli, the question arises whether the feeble response of PEC can be ascribed to the presence in the test population of an increased proportion of resident cells. Whereas this possibility cannot be ruled out as a contributing factor, it cannot in itself account for the low level of responsiveness observed. Evidence for this was secured in chemotaxis tests with mixtures of resident (nonresponsive) and recruited (responsive) macrophages from CONV donors. Mixed populations corresponding in their proportion to PEC populations from GB donors responded more vigorously than PEC from GB rats. It would seem, therefore, that the unresponsiveness of exudate macrophages from GB rats is related to an intrinsic dysfunction of the cells concerned.

The mechanism underlying the defect in GB macrophages is unknown. However, there are reasons for thinking that their failure to respond to chemotactic stimuli is related in some way to the metabolic activity of the cells, i.e., to their low level of "activation." It is in keeping with this view that highly activated macrophages from purified protein derivative-induced exudates borne by BCG-infected mice exhibit enhanced responsiveness to chemoattractants when compared with similarly elicited macrophages from uninfected donors (19). By using 10 macrophage-"activating" or -"stimulating" agents, Meltzer et al. (19) demonstrated a correlation between the chemotactic response of the cells and their tumoricidal capacity. The above findings encourage the speculation that the macrophages of GB rats are in a low state of activation and consequently respond poorly to chemotactic stimuli. Since *Shigella* endotoxin and lipid A are potent macrophage-activating agents (1), it is plausible that such microbial products derived from the intestinal flora and/or the environment have a role in the host's defense by stimulating macrophage metabolic activity and, hence, their chemotactic responsiveness.

However that may be, it has long been known that monocyte-derived macrophages have an indispensable role in the expression of DTH and cellular resistance to infection (16). Therefore it is logical to postulate that the diminished responsiveness of GB macrophages to chemotactic stimuli is causally related to the inability of flora-deprived animals to express DTH (14, 15, 32, 33) and their susceptibility to tuberculosis (31). There is evidence to support this proposition. Lev and Battisto (15) reported that lymphoid cells from specifically immunized guinea pigs can transfer tuberculin sensitivity to CONV recipients but not to guinea pigs that have been reared in a GF environment. Similar observations have been made in mice (32). Preliminary experiments performed in rats (Jungi and McGregor, unpublished data) indicate that labeled lymphoblasts from the thoracic duct lymph of *Listeria*-infected CONV donors can move in substantial numbers from the blood into *Listeria*-induced exudates borne by GF rats. The above finding takes on meaning when it is remembered that such animals can generate lymphocyte-dependent MCA, although their macrophages respond poorly to MCA in vitro (Table 3). These findings point to a normally functioning T-cell system and imply that the failure of GF animals to express DTH and cellular resistance to infection are related to the

TABLE 3. *Influence on exudate macrophages of lymphocyte-dependent MCA generated in the peritoneal cavity of either GF or CONV rats*[a]

Agent tested for MCA	Source of supernatant	Chemotactic activity[b]	
		Indicator macrophages from GB Fischer rats	Indicator macrophages from CONV LDA rats
L. monocytogenes-induced supernatants from specifically immunized rats	GF Fischer rats	4.3 ± 1.8	132.8 ± 25.1
	CONV LDA rats	11.6 ± 5.6	199.0 ± 30.0
L. monocytogenes-induced supernatants from normal non-immunized subjects	GF Fischer rats	0.1 ± 0.4	3.1 ± 1.8
	CONV LDA rats	0.9 ± 0.5	11.0 ± 3.7
Peritoneal supernatants from unstimulated *Listeria*-immune subjects	GF Fischer rats	0.4 ± 0.5	29.1 ± 22.6
	CONV LDA rats	ND[d]	16.4 ± 5.6
Heated rat serum (control)[c]		63.5 ± 11.6	294.0 ± 19.7

[a] Peritoneal cavities were washed with 3 ml of medium; cell-free supernatants were pooled within groups and tested for chemotactic activity. Indicator cells were peptone-elicited PEC.

[b] Cells per field (mean of 8 ± standard deviation).

[c] Heated for 30 min at 56°C.

[d] ND, Not done.

inability of the host to marshal monocyte-derived macrophages into lesions.

Whereas the macrophages of GB rats are severely impaired in their capacity to respond to chemoattractants, a comparable defect could not be demonstrated for neutrophils (Table 2). GB rats also marshaled into peptone-induced exudates more neutrophils than did their flora-bearing counterparts. The latter observation is at variance with a report by Trippestad and Midtvedt (30) who found a markedly diminished accumulation of neutrophils in casein-induced exudates. However, in accordance with our results, the above authors did observe that peritoneal exudate neutrophils from GB/GF rats respond in vitro to serum-derived factors in the manner of CONV donors.

Still other experiments reported here and elsewhere (30) failed to reveal an obvious difference in the intrinsic and induced chemotactic activity of sera from GB and CONV donors. These observations are important insofar as they point to an isolated defect in the responsiveness of GB macrophages.

Diminished responsiveness of macrophages to chemoattractants has been described in a variety of experimental circumstances. Keller and Sorkin (12) reported that neutrophils respond poorly to chemotactic stimuli after phagocytosis. A similar mechanism may account for the decreased responsiveness of rat peritoneal macrophages soon after i.p. injection of *L. monocytogenes* (Jungi, unpublished data). It also has been noted that neutrophils can be "deactivated" in vitro by incubating the cells in media containing high concentrations of chemoattractants (34). More recently, it has been reported that monocytes (13) and neutrophils (23) respond poorly or not at all to chemotactic stimuli after in vitro treatment with certain viruses. In these circumstances, however, neither has the mechanism of unresponsiveness been defined, nor has the behavior of the cells been related in a clear and unambiguous way to the level of macrophage activation as defined, for example, by metabolic parameters or the microbicidal activity of the cells concerned.

It is interesting in this connection that the mononuclear phagocytes of tumor-bearing subjects often are impaired in their responsiveness toward chemotactic agents (2, 20, 27, 28). Monocyte-derived macrophages have a decisive role in the destruction of certain tumors (3). Therefore, a dysfunction of macrophages, as expressed in diminished responsiveness toward chemotactic stimuli, might be expected to favor tumor growth. Studies reported by Meltzer (18) are pertinent in this connection. He demonstrated that the macrophages of GF nude mice are less aggressive toward tumor target cells than are the macrophages of CONV nude mice. This would be expected if a meaningful association

exists between the level of macrophage activation, the response of the cells to chemotactic stimuli, and their tumoricidal behavior in vitro. Since germ-free nude mice have a higher incidence of lymphoreticular tumors than do conventional controls (22), and since both groups lack T-cell functions, it is tempting to speculate that macrophage dysfunction could seriously compromise the host's defense against tumors, particularly under conditions of absent or impaired T-cell cytotoxicity.

The failure of GB macrophages to respond in vitro to chemotactic stimuli is interesting for another reason. Demonstration of a potentially reversible dysfunction of macrophages affords an opportunity to probe mechanisms underlying the induction of chemotactic responsiveness and possibly also the activation process in macrophages. Insight into these mechanisms might allow controlled modulation of macrophage functions in vivo.

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