イヌの肺胞マクロファージ画分の免疫学的機能における heterogeneity について

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Heterogeneity in Immunologic Functions among Canine Alveolar Macrophage Subfractions

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ABSTRACT. Canine alveolar macrophages (AM) were examined for the presence of subpopulations with differnt immunologic functions. Lavaged bronchoalveolar cells (BAC) from normal beagle dogs were separated into 3 density subfractions by centrifugation through discontinuous gradients of Percoll solution. After incubated on plastic plates, each of adherent BAC subfractions recovered was identified as AM by morphologic criteria and phagocytic capacity of latex beads. These adherent BAC subfractions were then incubated with lipopolysaccharides (LPS) and/or silica for testing their capacity to produce interleukin 1 (IL-1). IL-1 activity of culture supernatants was greater both in the lowest and intermediate density subfractions, but much lower in the highest density subfraction. When each of adherent BAC subfractions was incubated with autologous nylon wool-passed lymphocytes from tracheobronchial lymph nodes in the presence of a lectin mitogen, phytohemagglutinin (PHA), proliferative responses of lymphocytes were prominently enhanced by increasing cell numbers of adherent BAC subfractions. On the contrary, another mitogen, concanavalin A (Con A)-induced lymphocyte responses were suppressed by addition of both of the lowest and intermediate density subfractions, whereas only the highest density subfraction enhanced lymphocyte response. The suppressive effect of adherent BAC subfractions on Con A-induced responses was, however, inhibited by addition of indomethacin, prostaglandin synthesis inhibitor. These results indicate the presence of functional subpopulations among canine AM with respect to IL-1 production and accessory cell function for mitogen-induced lymphoproliferation.—KEY WORDS: alveolar macrophage, canine, heterogeneity, immunologic function.

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Macrophages are recognized to regulate lymphocyte response and present antigens in the immune system [19, 20]. Pulmonary alveolar macrophages (AM) have, therefore, an important role in pulmonary immunity against reespirable antigens including microorganisms [10, 13]. Many studies on AM, however, have suggested their capacity both to enhance and suppress immune responses in humans and several animal species [5, 7, 9, 11, 15, 21, 22]. Canine AM have been reported to act suppressively on mitogen-induced lymphocyte responses [1, 4]. It is not fully elucidated whether such differences in immunologic functions of AM may reflect interspecies differences [12] or the presence of heterogenous subpopulations with different functions [14, 23]. The present study was performed to investigate AM subpopulations of normal beagle dogs for their heterogeneity of immunologic functions. Our results show that AM subfractions isolated on the basis of cell density display different capacities for the production of monokine, interleukin 1 (IL-1) and for the effects on mitogen-induced lymphocyte proliferation. These findings can provide evidence for the presence of subpopulations of canine AM with heterogenous immunologic functions.

MATERIALS AND METHODS

Animals: A total of 16 (8 females and 8

males) nomal beagle dogs 1 to 3 years old were obtained from the breeding facility in our institute. All animals had no apparent pulmonary disorders by clinico-pathological inspections before and after experiments.

Bronchoalveolar lavage: After each animal was exsanguinated under the anesthesia with ketamine chloride (Ketalar®, Sankyo Co., Ltd.), bronchoalveolar lavage was performed aseptically by the repeated instillation of prewarmed phosphate buffered saline (PBS, pH 7.2) containing 100 μg/m*l* streptomycin and 100 U/m*l* penicillin through a cannulated silicon tube. After washing three times with serum-free RPMI-1640 medium (Flow Laboratory, McLean, VA), containing 100 μg/m*l* streptomycin and 100 U/m*l* penicillin, bronchoalveolar cells (BAC) were resuspended in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS; GIBCO, Grand Island. NY).

Preparation of BAC subfractions: For isolation of different density subfractions, Percoll® (Pharmacia Fine Chemicals, Uppsala, Sweden) was diluted from 100% stock with sterile PBS (pH 7.2) to strengths of 45, 55 and 65%. Three-step discontinuous gradients were then prepared by sequentially layering 2-m*l* volumes of 65–45% solution into a centrifuge tube. Each of 2-m*l* BAC suspension was thereafter layered upon the top (45%) layer, and the tube was centrifuged at 400 g for 30 min at 20°C. The cells were harvested by carefully collecting through pasteur pipetts, and three fractions collected (I-III). The density of each fraction was estimated by standardization with density marker beads (Pharmacia). These fractions were washed three times, resuspended in RPMI-1640 medium supplemented with 10% FCS, and used for the following experiments.

Morphologic identification of BAC subfractions: Each of BAC subfractions isolated by Percoll gradients was tested for their morphology by Giemsa staining, α-naphtyl-acetate esterase (ANAE) content using a commercial kit (Sigma Chemical Co., St. Louis, Mo), phagocytosis of latex particles, trypan blue dye exclusion, and for the cell number by Coulter counter (Model Zf; Coulter Electronics, Hialeah, FL). The capacity to adhere on a plastic plate was also examined in each of BAC subfractions. After incubating each cell suspention on the plastic petri dish (Falcon 1007, Becton Dickinson Labware, Oxnard, CA) for 2 hr at 37°C in a humidified environment of 5% CO_2 in air, non-adherent cells were removed by washing. The adherent BAC subfractions were then also tested for their morphology by the above-described criteria after scraping them with a rubber policeman.

Separation of canine lymphocytes: In this study, canine lymphocytes were prepared from tracheobronchial lymph nodes (TBLN) after a bronchoalveolar lavage procedure. Each of TBLN from beagles was cut into small pieces with a sharp razor, minced on a wire screen mesh in RPMI-1640 medium, washed three times, and resuspended in FCS-containing medium. These lymph node lymphocytes (whole LN cells) contained 20–30% of macrophages by morphological criteria as described avove. For further purification of lymphocytes [1], whole LN cells were layered upon a packed nylon wool column and incubated for 2 hr at 37°C. Non-adherent, nylon wool-passed cells were then collected, washed, and resuspended in FCS-containing medium. These nylon wool-passed lymph node cells (NWLN cells) contained 10% or less of macrophages. Either whole LN cells or NWLN cells were used for the following cell cultures.

Cell cultures for IL-1 production: Adherent BAC subfractions were adjusted to a concentration of 5×10^5 cells/m*l*. One milliliter of each adherent subfraction was deli-

vered to 24-flat-bottom well tissue culture plates (Falcon 3047), and 1.0 m*l* of medium alone or medium containing 250 μg/m*l* silica (Sigma) and/or lipopolysaccharides (LPS) from *E. coli* 0.55:B5 (Difco, Detroit, MI) was added to each well for the induction of IL-1 production [6]. Cultures were then incubated for 24 hr at 37°C in a humidified environment of 5% CO_2 in air. Each of culture supernatants (AM-SUP) was harvested, and stored at −80°C until used.

IL-1 activity of these AM-SUP was tested by measuring their capacity to co-stimulate proliferative response of mouse thymocytes in the presence of submitogenic dose of mitogen as described previously [17]. Briefly, 1.5×10^6 thymocytes from C3H/HeJ mice were incubated with AM-SUP at a final dilution of 1:4 in the presence of 1.0 μg/m*l* phytohemagglutinin (PHA; Sigma) for 72 hr, and pulsed with 0.5 μCi of tritiated thymidine (^{3}H-TdR; 5 Ci/mmol, Amersham, UK) for the last 8 hr of cultures. After harvesting cells, ^{3}H-TdR incorporation was counted by a liquid scintillation spectrometer (Beckman LS7500).

Cell cultures for mitogen-induced lymphocyte proliferation: The concentration of adherent BAC subfractions was adjusted to $1\times10^4-2\times10^5$ cells/m*l*. A 0.1 m*l* aliquot of subfractions at each concentration was delivered to each well of 96-well microculture plates (Falcon 3072). After cultures of BAC subfractions were irradiated with 2000 R of X-ray (Shimazu), 0.1 m*l* aliquots of autologous lymphocytes (1×10^6/m*l*) from either whole LN cells or NWLN cells preparations were added to the cultures with 10 μg/m*l* of PHA or concanavalin A (Con A; Sigma), incubated for 72 hr, and pulsed with 0.5 μCi of ^{3}H-TdR for the last 8 hr of incubation. The uptake of ^{3}H-TdR by lymphocytes was counted as above described.

RESULTS

Beagle BAC were divided into 3 subfractions by centrifugation through Percoll discontinuous gradients. The lowest density (1.032–1.046 g/m*l*) subfraction, I, contained the majority of total cells recovered, whereas both the intermediate density (1.046–1.061 g/m*l*) subfraction, II, and the highest density (1.061–1.074 g/m*l*) subfraction, III, contained only about 10–20% of cells recovered (Table 1). By Giemsa staining, the proportion of AM in each of BAC subfractions was higher in subfractions, I and II, and was only about 50% in subfraction, III, in which about 50% of cells were identified as lymphocytes. The proportion of polymorphonuclear leukocytes (PMN) was very low in each of BAC subfractions. Non-specific esterase (ANAE)-positive cells in each of BAC subfractions were less than 50% in total cells, while the proportion of phagocytic cells was higher in the lowest density subfraction, I, about 50% in the intermediate density subfraction, II, and very low in the highest density subfraction, III, respectively.

After these BAC preparations were incubated on plastic plates for 2 hr, most of adherent cells recovered in each of subfractions exhibited to contain high proportions (95% or more) of phagocytic cells by latex beads-ingestion test (Table 2). These adherent BAC subfractions were, therefore, used for the following experiments as AM sources from BAC, together with unfractionated adherent BAC preparations.

When an equal number (5×10^5) of each of adherent BAC preparations was incubated *in vitro* for 24 hr in the absence of stimulants, IL-1 activity of the supernatants (AM-SUP) detected by co-mitogenic responses of mouse thymocytes was very low

Table 1. Subfractions of beagle bronchoalveolar cells (BAC) isolated by centrifugation through Percoll discontinuous density gradients

	BAC[a] subfraction (Density Range, g/ml)			
	Unfractionated	I (1.032–1.046)	II (1.046–1.061)	III (1.061–1.074)
Total Number of Cells ($\times 10^{-7}$)	5.5± 4.5[e]	4.6± 3.8	0.74± 0.73	0.15± 0.13
Total Cells Recovered (%)	100	87.3±13.9	10.2 ±10.1	2.4 ± 2.1
Morphology (Giemsa, %)				
AM[b]	70.1± 9.3	74.6± 5.7	65.0 ± 9.9	45.9 ±13.4
Lymphocyte	25.3± 9.2	20.6± 5.6	29.9 ±11.0	49.4 ±13.2
PMN[c]	4.8± 3.5	4.9± 4.2	5.0 ± 3.7	4.6 ± 4.0
ANAE[d] (%)	49.9± 5.7	48.1±11.6	40.9 ±10.5	31.0 ± 7.2
Latex-Ingestion (%)	71.7±13.6	73.1±12.5	45.9 ±15.2	17.4 ± 8.6

a) bronchoalveolar cell.
b) alveolar macrophage.
c) polymorphonuclear leukocyte.
d) α-naphtyl acetate esterase.
e) Mean±SD of 16 beagles.

Table 2. Enrichment of alveolar macrophages in adherent BAC subfractions

Adherent BAC subfractions	Viability (%)[a]	Latex-ingestion (%)[b]
Unfractionated	97.8±1.2[c]	95.4±1.6
I	97.6±1.3	95.7±2.0
II	97.4±2.0	96.4±1.2
III	97.0±0.8	92.5±1.0

a) Calculated with trypan blue dye exclusion test.
b) Counted phagocytic cells after incubated with 1.0% latex.
c) Data are expressed as mean±SD of 16 beagle dogs.

or undetectable in unfractionated and fractionated adherent BAC (Fig. 1A). IL-1 level was, however, elevated in cultures of unfractionated and both of fractionated adherent BAC, I and II, if LPS (50 μg/ml) was added, whereas only subfraction, III showed a low activity (Fig. 1B). When each of adherent BAC preparations was incubated together with LPS (50 μg/ml) and silica particles (250 μg/ml), IL-1 activity was greatly enhanced (Fig. 1C). Unfractionated and both of fractionated adherent BAC, I and II, exhibited higher IL-1 activity, although IL-1 level of subfraction, III was much lower, compared to the other subfractions and unfractionated adherent BAC.

These results indicate that the capacity of IL-1 production by stimulation of LPS and/or silica is different among AM subfractions, and that the highest density subfraction of adherent BAC may have a lower potential for IL-1 production.

The effect of adherent BAC subfractions as well as unfractionated preparations, was tested for their accessory cell function on PHA-induced response of LN cells. When unfractionated or fractionated adherent BAC at an increasing cell number (1×10^3 to 2×10^4 /well) were added after X-irradiation to autologous whole LN cells (1×10^5 /well) and incubated for 72 hr in the presence of 10 μg/ml PHA, ^{3}H-TdR incorporation by LN cells was expressed as a percentage of the control response of LN cells alone, which showed 6,000 to 8,000 cpm (Fig. 2A). The PHA-induced responses of whole LN cells were enhanced almost twice as the control response by increasing cell numbers of unfractionated and each of fractionated adherent BAC. If each of these adherent

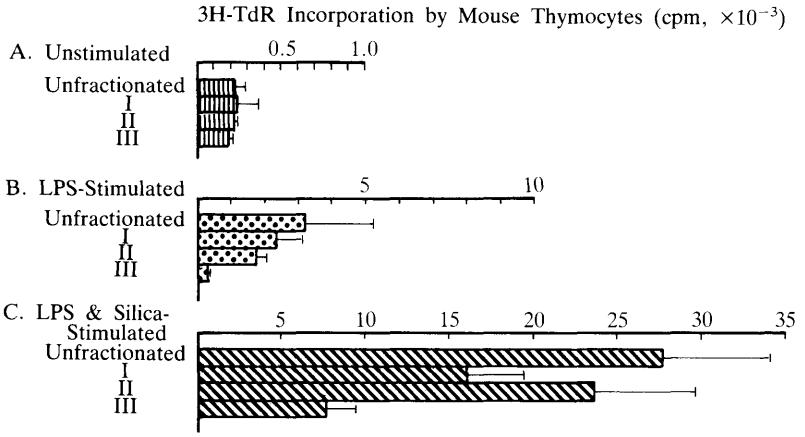

Fig. 1. IL-1 activity of culture supernatants from unfractionated and fractionated adherent BAC prepatrations. Each of adherent BAC (5×10^5) was incubated for 24 hr either in the absence of stimulants (A) or in the presence of 50 μg/ml LPS alone (B) or both of 50 μg/ml LPS and 250 μg/ml silica (C). Each of supernatants was tested for IL-1 activity by ^{3}H-TdR incorporation of mouse thymocytes co-stimulated with a suboptimal dose (1 μg/ml) of PHA. The data are expressed as mean±SD of experiments of 16 beagles.

BAC preparations was similarly added to autologous NWLN cells (1×10^5 /well), which alone showed lower (300–1,000 cpm) response in the presence of PHA, the responses of NWLN cells were more prominently enhanced by increasing cell numbers of unfractionated and fractionated adherent BAC (Fig. 2B). However, only subfraction, III which also enhanced PHA-induced response of NWLN cells in a dose-dependent manner, was likely to support lymphocyte response at a lesser degree than the other subfractions, I and II.

The effect of adherent BAC subfractions was also tested for their accessory cell function on another lectin mitogen, Con A-induced response of LN cells. Whole LN cells (1×10^5 /well) alone showed ^{3}H-TdR incorporation in the range of 23,000 to 38,000 cpm after 72 hr-incubation in the presence of 10 μg/ml Con A (100% as the control response). When unfractionated or fractionated adherent BAC were added after X-irradiation to these whole LN cells, the Con A-induced lymphocyte responses were suppressed by increasing cell numbers of each of adherent BAC preparations (Fig. 3A). The Con A-induced responses of NWLN cells, which alone showed lower ^{3}H-TdR incorporation (3,000–9,000 cpm), were, however, slightly enhanced by addition of smaller numbers of unfractionated and both of fractionated adherent BAC, I and II (Fig. 3B). Only the addition of subfraction, III, enhanced Con A-induced response of NWLN cells in a dose-dependent manner.

These findings indicate that the effect of adherent BAC subfractions on Con A-induced lymphoproliferation of canine LN cells may be different from that on PHA-induced responses.

Since prostaglandin-E series (PGE) generated by adherent cells could suppress Con A-induced lymphocyte responses in the dog [1, 4], indomethacin, a PGE synthesis inhibitor, was added to co-cultures of NWLN cells and autologous adherent BAC preparations (Fig. 4). Although NWLN cells alone (1×10^5 /well) showed a lower ^{3}H-TdR incorporation (4,000–5,000 cpm) whether or not in the presence of 1.0 μg/ml

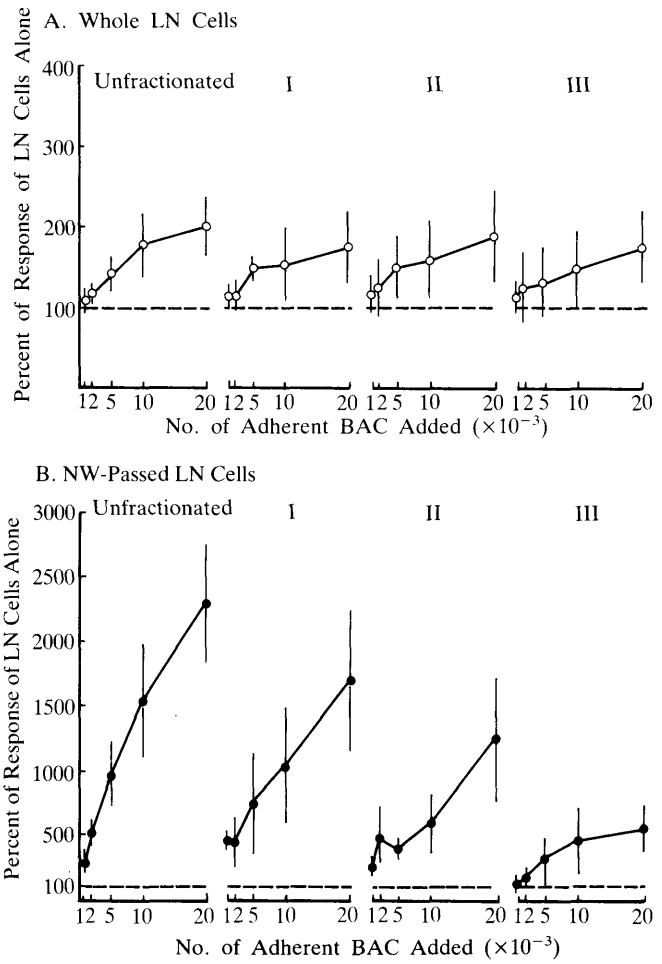

Fig. 2. Effect of unfractionated and fractionated adherent BAC preparations on PHA-induced response of LN cells. Cells at a increasing number (1×10^3 to 2×10^4) from each of adherent BAC preparations were added to 1×10^5 of whole LN cells (A) or NWLN cells (B), and incubated for 72 hr in the presence of 10 μg/ml PHA. The response of LN cells, measured as ^{3}H-TdR incorporation, was calculated as a percentage of response of LN cells alone (6,000 to 8,000 cpm for whole LN cells and 300–1,000 cpm for NWLN cells). The data are expressed as mean±SD of experiments of 8 beagles.

indomethacin, suppression of Con A-induced responses by adherent BAC preparations was prominently inhibited by addition of indomethacin, and lymphocyte responses were rather enhanced by increasing cell numbers of unfractionated and each of fractionated adherent BAC. The result, therefore, suggests PGE-mediated suppression by adherent BAC subfracitons on Con A-induced response of LN cells.

DISCUSSION

The purpose of this study was to confirm the presence of heterogenous AM subfractions with different immunologic functions in the dog, since the detailed features are

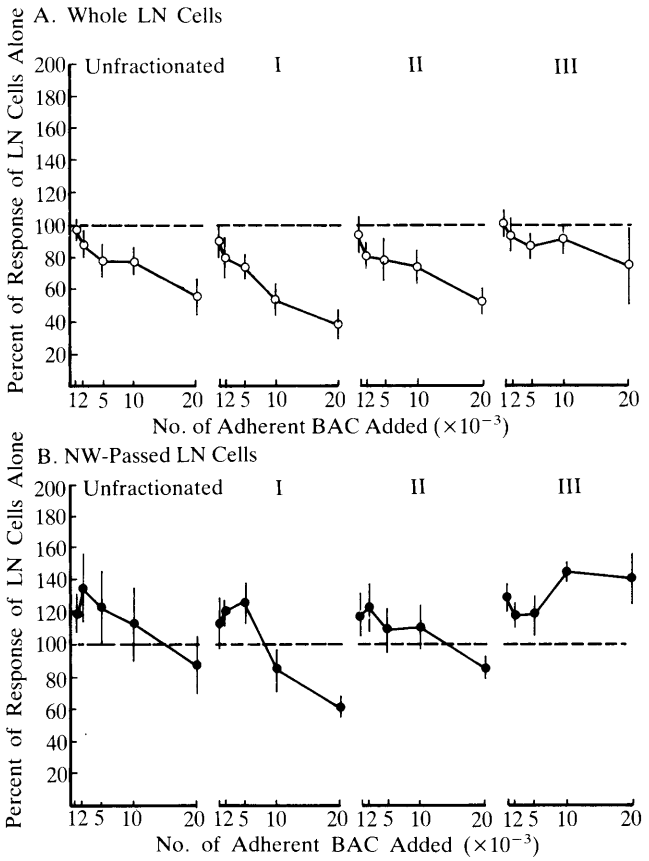

Fig. 3. Effect of unfractionated and fractionated adherent BAC preparations on Con A-induced response of LN cells. Cells at a increasing number (1×10^3 to 2×10^4) from each of adherent BAC preparations were added to 1×10^5 of whole LN cells (A) or NWLN cells (B), and incubated for 72 hr in the presence of 10 μg/ml Con A. The response of LN cells, measured as ^{3}H-TdR incorporation, was calculated as a percentage of recponse of LN cells alone (23,000 to 38,000 cpm for whole LN cells and 3,000–9,000 cpm for NWLN cells). The data are expressed as mean±SD of experiments of 8 beagles.

not fully elucidated especially on canine lung macrophages and their role in pulmonary immunity. Three different density BAC subfractions could be isolated by centrifugation through Percoll gradients, and at least on the morphologic basis, they displayed heterogenous constituents. Majority of cells recovered was concentrated in the lowest density BAC subfraction, I, while cell numbers in the highest density subfraction, III, were much fewer. The proportion of AM or phagocytic cells was higher both in the lowest and intermediate density subfractions, I and II, but much lower in the highest density subfraction, III. These findings suggest that majority of canine AM may have normally lower density, compared to other species [3, 23]. As more than 95% of adherent cells were phagocytes in each of BAC subfractions after they were incubated on plastic plates, we used these adherent BAC preparations as AM subfractions.

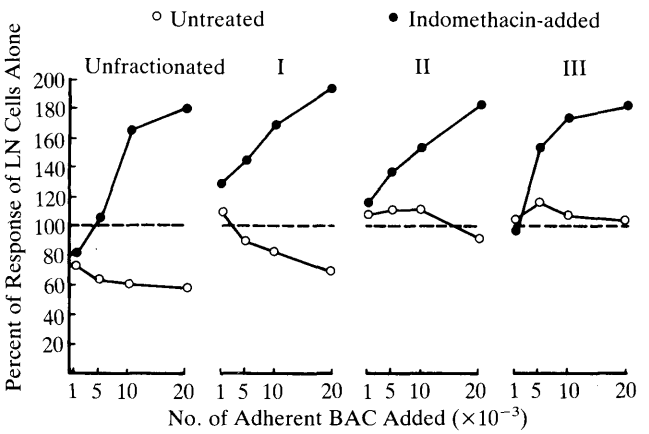

Fig. 4. Inhibition by indomethacin of suppressive effects of unfractionated and fractionated adherent BAC preparations on Con A-induced response of LN cells. Cells at a increasing number (1×10^3 to 2×10^4) from each of adherent BAC preparations were added to 1×10^5 of NWLN cells in the absence or presence of 1.0 μg/m*l* indomethacin, and incubated for 72 hr with 10 μg/m*l* Con A. The response of LN cells, measured as ^{3}H-TdR incorporation, was calculated as a percentage of response of LN cells alone (4,000 to 5,000 cpm both for untreated or indomethacin-added cultures). The data are expressed as mean values of experiments of 2 beagles.

IL-1 production by unstimulated AM from normal beagles was not noted, whereas AM incubated with LPS and /or silica particles can produce IL-1 at a higher level as reported previously [17]. In the present study, the capacity of IL-1 production was also much lower in each of adherent BAC subfractions as well as unfractinated adherent BAC under unstimulated condition. Both of LPS- and/or silica-stimulated subfractions, I and II, however, generated higher IL-1 activity, while only subfraction, III, showed much lower capacity to produce IL-1 even if it was stimulated with LPS and silica. These results indicate different potentials for IL-1 production among canine AM subpopulations.

The present study also provides important data showing heterogenous effects of AM subfractions on mitogen-induced lymphoproliferation. It has been recognized that canine pulmonary AM suppress lymphocyte responses under stimulation of a variety of lectin mitogens [1, 4]. Our results were, however, that PHA-induced lymphoproliferation was enhanced by addition of adherent BAC preparations, whereas Con A-induced response was suppressed by adherent BAC. The enhancement of PHA-induced response of NWLN cells was more prominent by addition of both of the lowest and intermediate density subfractions, I and II, than the highest density subfraction, III, which showed less supportive effect on lymphocyte response. On the contrary to this, Con A-induced response of NWLN cells was enhanced only by the highest density subfraction, III, but was suppressed by both of the lowest and intermediate density subfractions, I and II, in a dose-dependent manner. This suppressive effect of AM on Con A-induced lymphoproliferation was found due to prostaglandins (PGE) probably produced by AM themselves, since indomethacin completely inhibited this suppressive effect. Although PGE-mediated immunosuppression by canine AM has been demonstrated on both Con A-

and PHA-induced lymphocyte responses by other studies [1, 4], the present data suggest in this regard that different mechanisms may be related to canine lymphocyte responses induced by PHA.

It was also shown in this study that the above-described effect, accessory cell function, of AM on lectin-induced lymphoproliferation may be likely independent on the capacity of IL-1 production among adherent BAC subfractions. Despite that both of the lowest and intermediate density subfractions, I and II, were capable to generate higher IL-1 activity, they could not support Con A-induced lymphoproliferation. On the contrary to this, the highest density subfraction, III, could support Con A-induced lymphocyte response in spite of its lower potentials for IL-1 production. On the other hand, the enhancement of PHA-induced lymphocyte responses by both of subfractions, I and II, may be due to their higher IL-1 generation. There remains, however, a considerable controversy on the role of IL-1 on lectin- or antigen-induced lymphocyte activation. Some reports indicate the requirement of IL-1 [16, 18], while others describe no IL-1 requirement for T cell activation [2, 8]. The different accessory cell function of AM subfractions in this study cannot be, therefore, explained at least only by the concept for lymphocyte activation through interleukins [19, 24]. In this regard, further characterization should be needed for canine AM subpopulations as well as more detailed fractionation.

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REFERENCES

1. Ansfield, M. J., Kaltreider, H. B., Caldwell, J. I., and Herskowitz, F. N. 1979. Hyporesponsiveness of canine bronchoalveolar lymphocytes to mitogens: inhibition of lymphocyte proliferation by alveolar macrophages. *J. Immunol.* 122: 542–548.
2. Chu, E., Rosenwasser, L. J., Dinarello, C. A., Lareau, M., and Geha, R. S. 1984. Role of interleukin 1 in antigen-specific T cell proliferation. *J. Immunol.* 132: 1311–1316.
3. Dauker, J. H., Holian, A., Rosemiller, M. E., and Daniele, R. P. 1983. Separation of bronchoalveolar cells from the guinea pig on continuous density gradients of Percoll: morphology and cytochemical properties of fractionated lung macrophages. *J. Reticuloendothel. Soc.* 33: 119–126.
4. Demenkoff, J. H., Ansfield, M. J., Kaltreider, H. B., and Adam, E. 1980. Alveolar macrophage suppression of canine bronchoalveolar lymphocytes: the role of prostaglandin E_2 in the inhibition of mitogen -responses. *J. Immunol.* 124: 1365–1370.
5. Ettensohn, D. B., and Roberts, N. J., Jr. 1983. Human alveolar macrophage support of lymphocyte respenses to mitogens and antigens. Analysis and comparison with autologous peripheral blood-derived monocytes and macrophages. *Am. Rev. Resp. Dis.*: 516–522.
6. Gery, I., Davies, P., Derr, J., Krett, M., and Barranger, J. A. 1981. Relationship between production and release of lymphocyte activating factor (interleukin 1) by murine macrophages I. Effects of various agents. *Cell. Immunol.* 64: 293–303.
7. Gorenberg, D. J., and Daniele, R. P. 1978. The alveolar macrophages: its capacity to act as accessory cell in mitogen-stimulated proliferation of guinea pig lymphocytes. *Cell. Immunol.* 36: 115–127.
8. Haq, A. U., Mayernik, D. G., Orosz, C., and Rinehart, J. J. 1984. Interleukin 1 secretion is not required for human macrophage support of T-cell proliferation. *Cell. Immunol.* 87: 517–527.
9. Herscowitz, H. B., Conrad, R. E., and Pennline, K. J. 1979. Alveolar macrophage-induced suppression of the immune response. *Am. Exp. Med. Biol.* 121: 459–484.
10. Hocking, W. G., and Gold, D. W. 1979. The pulmonary alveolar macrophage. *N. Engl. J. Med.* 301: 639–645.
11. Holt, P. G. 1979. Alveolar macrophages. II. Inhibition of lymphocyte proliferatioln by purified macrophages from rat lung. *Immunol.* 37: 429–436.
12. Holt, P. G. 1980. Alveolar macrophages, IV. Interspecies differences in activity in proliferating lymphocyte cultures. *Cell. Immunol.* 50: 210–215.
13. Hunninghake, G. W. Gadek, J. E., Kawanami,

O., Ferrans, V. J., and Crystal, R. G. 1979. Inflammatory and Immunoprocesses in the human lung in health and disease: evaluation by bronchoalveolar lavage. *Am. J. Pathol.* 97: 149–206.
14. Kaltreider, H. B. 1982. alveolar macrophages: enhancers or suppressors of pulmonary immune rectivity? *Chest.* 82: 261–262.
15. Liu, M. C., Proud, D., Schleimer, R. P., and Plaut, M. 1984. Human lung macrophages enhance and inhibit lymphocyte proliferation. *J. Immunol.* 132: 2895–2903.
16. Maizel, A. L., Mehta, S. R., Ford, R. J., and Lachman, L. B. 1981. Effect of interleukin 1 in human thymocytes and pufified human T cells. *J. Exp. Med.* 153: 470–475.
17. Oghiso, Y., Kubota, Y., Fukuda, S., and Haruzo, I. 1985. Interleukin 1 released from beagle alveolar macrophages exposed to dust particles. *Jpn. J. Vet. Sci.* 47: 851–854.
18. Palacios, R. 1982. Concanavalin A triggers T lymphocytes by directly interacting with their receptors for activation. *J. Immunol.* 128: 337–342.
19. Rosenstreich, D. L. 1981. The macrophages *in* Cellelar Functions in Immunity and Inflammation; Ed-in-chief, J. J. Oppenheim, D. L. Rosensreich., and M. Potter, Elsevier/North-Holland, NY & Amsterdam. pp. 127–159.
20. Rosenstreich, D. L., and Mizel, S. B. 1978. The participation of macrophages and macrophage cell lines in the activation of T lymphocytes by mitogens. *Immunol. Rev.* 40: 102–135.
21. Rossi, G. A., Zocchi, E., Sacco, O., Balbi, B., Ravazzoni, C., and Damiani, G. 1986. Alveolar macrophage stimulation of T-cell proliferation in autologous mixed lymphocyte reactions. *Am. Rev. Resp. Dis.* 133: 78–82.
22. Sestini, P., Tagliabue, A., Boraschi, D. 1984. Modulation of macrophage suppressive activity and prostaglandin release by lymphokines and interferon: comparison of alveolar, pleural, and peritoneal macrophages. *Clin. Exp. Immunol.* 58: 573–580.
23. Shellito, J., and Kaltreider, H. B. 1984. Heterogeneity of immunologic function among subfractions of normal rat alveolar macrophages. *Am. Rev. Resp. Dis.* 129: 747–753.
24. Smith, K. A. 1980. T-cell growth factor. *Immunol. Rev.* 51: 337–357.

要　約

イヌの肺胞マクロファージ画分の免疫学的機能における heterogeneity について：小木曽洋一・久保田善久（放射線医学総合研究所内部被ばく研究部）――健常ビーグル成犬から得た肺洗浄細胞を Percoll 不連続密度勾配重層遠沈により３つの画分に分離し，これらの各画分のプラスチックプレート付着性肺胞マクロファージ（AM）を LPS あるいはシリカ粒子で刺激・培養すると，低密度画分 AM が高いインターロイキン１（IL－１）活性を示した。一方，マイトジェンによる気管支リンパ節リンパ球の幼若化応答に対する各画分の効果を検討したところ，PHA ではすべての AM が増強効果を，Con A では高密度画分 AM を除き抑制効果を示した。抑制効果は，インドメサシン添加により阻止されたことから，プロスタグランジン E によるものと考えられた。以上からイヌの肺胞マクロファージに免疫学的機能の異なる亜群の存在が示唆された。