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Granuloma Formation by Artificial Microparticles *In Vitro*

Macrophages and Monokines Play a Critical Role in Granuloma Formation

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To investigate the basic mechanisms of granuloma formation, in vitro ***granulomas were induced by culturing murine spleen cells in the presence of artificial microparticles. Large granulomas developed around dextran beads. The lesions were inducible by spleen cells from either normal mice or athymic nude mice. Minimal inflammation was produced around latex beads. The histologic features and time kinetics of granulomas*** in vitro ***were well correlated with granulomas*** in vivo. ***Culture supernatants of dextran induced granulomas contained high levels of interleukin-1 (IL-1) activity but not interleukin-2 (IL-2) or interleukin-4 (IL-4) activity. IL-1 activity was correlated with granuloma size. Additionally, granulomas were produced by culturing spleen cells in the presence of agarose beads coupled to recombinant IL-1 or recombinant tumor necrosis factor-α (TNF-α). Granulomatous lesions also were induced by macrophage-enriched populations in the presence of monokine-coupled beads. Adherent macrophages, but not nonadherent cells, were required for induction of granulomas*** in vitro. ***In contrast, very small lesions were seen when spleen cells or adherent cells were cultured in the presence of beads coupled to recombinant IL-2 or recombinant interferon-γ (IFN-γ). These results suggest that macrophages and monokines such as IL-1 and TNF-α play an essential role in granuloma formation*** in vitro. ***(Am J Pathol 1989, 134:1189–1199)***

Granulomas are focal, predominantly mononuclear tissue inflammations evoked by persistent irritants.[1,2] Granulomatous inflammation is associated with many significant human diseases including tuberculosis, leprosy, sarcoidosis, schistosomiasis, berylliosis, and zirconium granulomas. Little information is available currently concerning the mechanism of granuloma formation. In view of the predominant participation of macrophages in granulomas, it is reasonable to assume that macrophages and their products may be involved in the development of granulomatous inflammation. Monokines such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) are produced primarily by monocytes/macrophages and these are known to be responsible for inflammatory and immunologic events.[3–6] In fact, our recent studies have shown that IL-1 plays a role in the initiation and development of experimental pulmonary granulomas in mice.[7–10]

To clarify the fundamental mechanism of granuloma formation, *in vitro* granulomas were induced by culturing immunocompetent cells with artificial microparticles. The present report gives evidence that *in vitro* granulomas are inducible by culturing murine spleen cells in the presence of artificial microparticles. The kinetics of the lesions was well correlated with monokine activity in the culture supernatants. Additionally, *in vitro* granulomas were produced by culturing spleen cells in the presence of agarose beads coupled to monokines (IL-1 or TNF-α), but not T cell-derived lymphokines (interleukin-2 [IL-2] or interferon-γ [IFN-γ]). Macrophages were necessary for granuloma formation induced by dextran beads and monokine-coupled beads. These results suggest that macrophages and monokines such as IL-1 and TNF-α play a critical role in granuloma formation *in vitro*.

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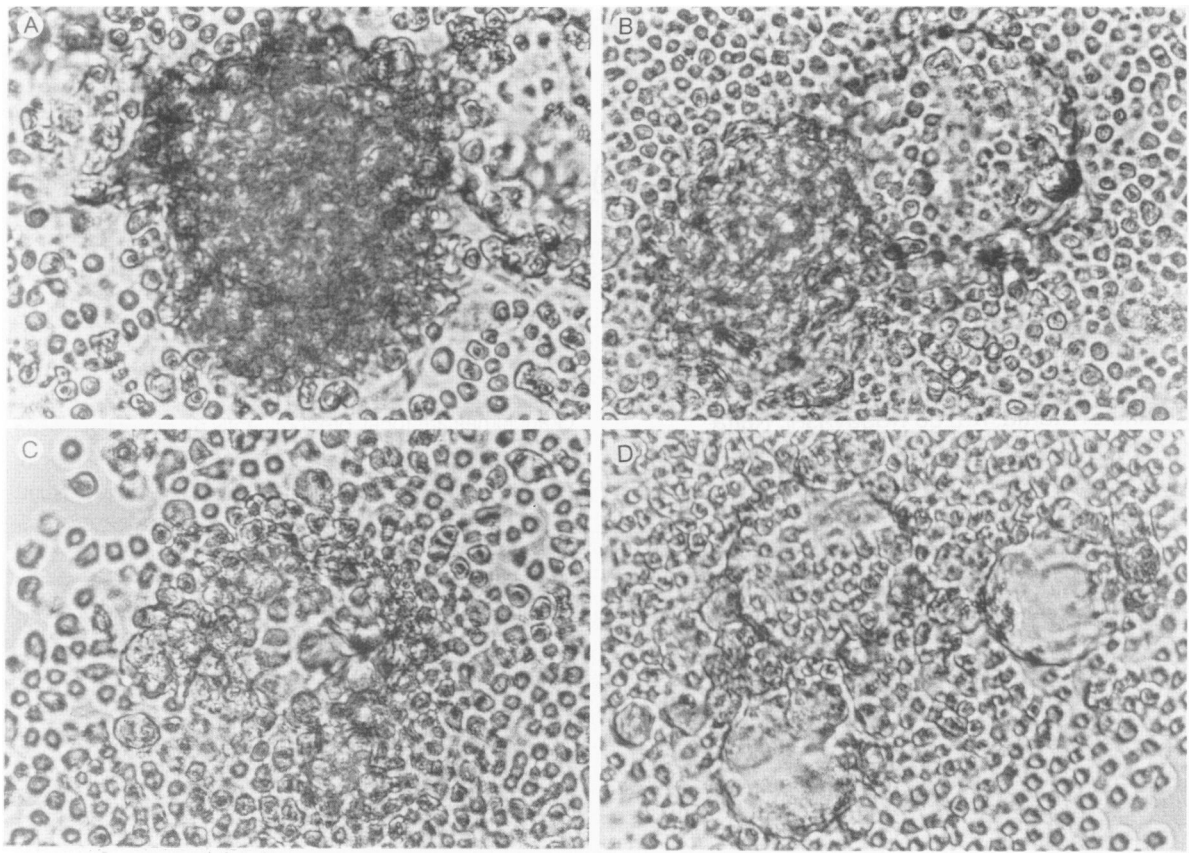

Figure 1. *Granulomatous reactions of spleen cells to dextran beads* in vitro. *Multiple spleen cells are bound to beads. Spleen cells were obtained from Balb/c* **(A)**, *C57BL/6* **(B)**, *C3H/He* **(C)**, *and athymic nude mice* **(D)**. *The lesions are shown 3 days after the culture (×150). The granulomatous reactions induced by dextran beads are composed predominantly of macrophages with fewer neutrophils and lymphocytes.*

Materials and Methods

Mice

Female Balb/c (H-2^d), C57BL/6 (H-2^b), C3H/He (H-2^k) and congenitally athymic Balb/c nude (nu/nu) mice were purchased from Charles River Japan, Tokyo. All mice were used at 6 to 9 weeks of age.

Culture Medium

Cells were cultured in RPMI 1640 supplemented with L-glutamine (2 mM), gentamycin (50 μg/ml, GIBCO, Grand Island, NY), 2-mercaptoethanol (5×10^{-5} M), and 10% heat-inactivated fetal bovine serum (GIBCO). This medium will be referred to as complete culture medium.

Induction of Granulomas In Vitro

Spleen cells were cultured in complete culture medium in the presence of artificial microparticles (70 to 100 μ in diameter) such as dextran beads (Sephadex G-50; Pharmacia Fine Chemicals, Piscataway, NJ), polystyrene beads (latex; Polysciences Inc., Warrington, PA) or cytokine-coupled cyanogen-bromide activated agarose beads (Sepharose 4B; Pharmacia Fine Chemicals). In certain experiments, artificial beads were incubated with thymocytes instead of spleen cells. Dextran and polystyrene beads were sterilized by autoclaving. Cytokine-coupled agarose beads were sterilized by incubating for 30 minutes at room temperature with 1% formaldehyde in phosphate-buffered saline (PBS) and extensively washed with sterile PBS.[11] The final culture contained 3×10^6 cells and 3×10^3 beads in 1.5 ml. The cells were cultured at 37 C in 5% CO_2. This dose had been shown previously to result in optimal responses. Cell-to-bead interactions were classified according to the methods described previously[12]: 1, no reaction; 2, 1 or more cells adhering to the bead; 3, 1 or more cells demonstrating the morphologic changes of blast formation either in the immediate vicinity of or directly adhering to the bead, accompanied by a circumoval mononuclear cell migration; 4, 1 adherent layer of cells surrounding the entire bead associated with increased

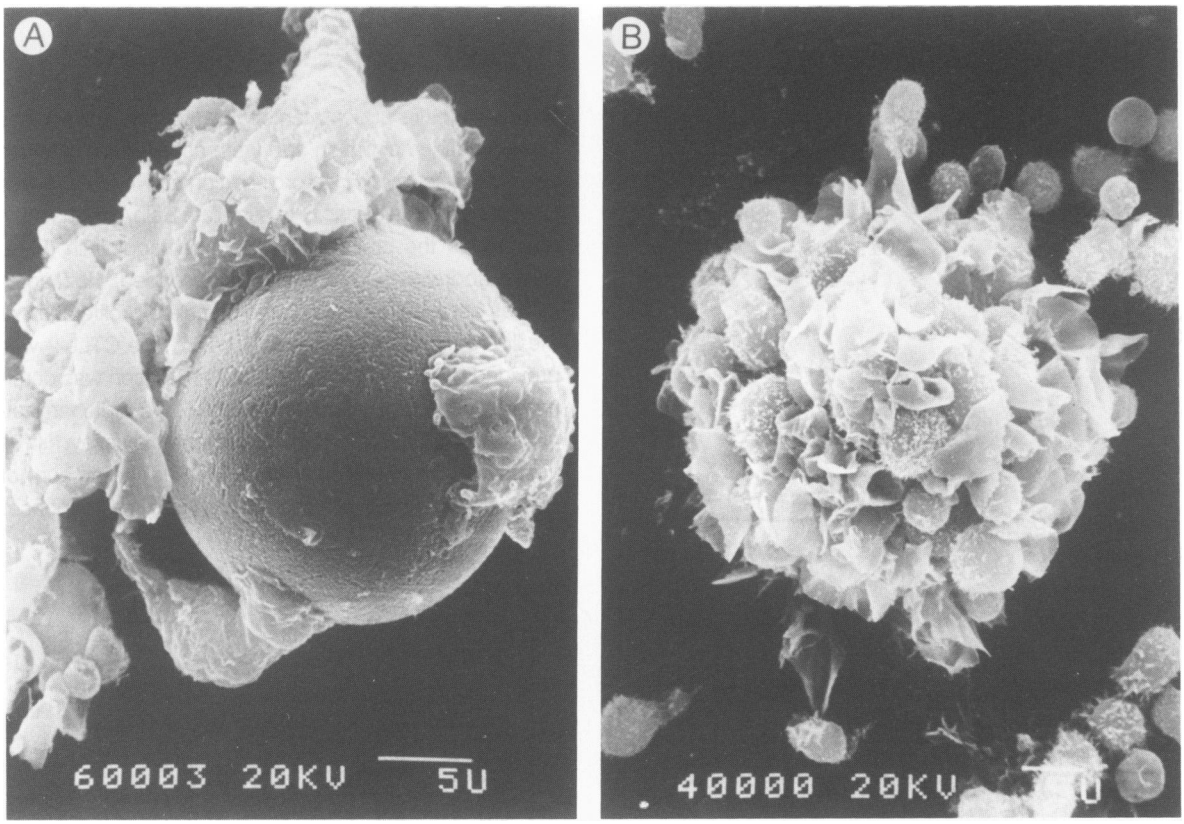

Figure 2. *Scanning electron micrographs of dextran-induced granulomas* in vitro. *Representative lesions seen 1 (A, ×3000) and 3 (B, ×2000) days after the culture of Balb/c mouse spleen cells in the presence of dextran beads. Macrophages attached to the bead surface are the predominant cell type.*

mononuclear cell migration; 5, multiple layers of adherent cells about the bead, accompanied by mononuclear cell migration. Beads 70 to 100 μ in diameter were selected for measurement. One hundred determinations were made for each culture well and each experimental group was studied in triplicate. Determinations were made using an inverted phase-contrast microscope (IMT-2, Olympus Co., Ltd., Tokyo, Japan). After classification, each bead reaction received the accompanying numerical score. The mean reaction for each experimental group was determined by multiplying the number of cell-to-bead reactions assigned to each of five categories by the number of that particular classification and then summing the total. The results are expressed as the resultant mean value of the triplicate and has been called the granuloma index (GI).

Electron Microscopy

At successive intervals after the addition of spleen cells and after the initiation of granuloma formation, the cultures were fixed *in situ* in 2.5% phosphate-buffered glutaraldehyde. They were postfixed in 2% OsO_4, dehydrated, and embedded in the epoxy resin. After polymerization, the thin plastic sheet was removed from the dish. The cultures were fixed as described above, dehydrated successively to absolute ethanol, coated with Au/Pd, and examined in an S700 scanning electron microscope (Hitachi Co., Ltd., Tokyo, Japan) operated at 20 kV.

Cell Separation

Spleen cells (2×10^7 cells) were incubated on a plastic petri dish at 37 C for 2 hours in 5% CO_2. The nonadherent cells were poured off and the residual nonadherent cells were removed by washing with warm complete culture medium. Approximately 6×10^6 adherent cells/dish remained (30% yield). Purity of adherent cell preparations was more than 95% macrophages, examined by nonspecific esterase staining.[13]

Cytokines

Recombinant human IL-1β, with a specific activity of 2×10^7 U/mg, was provided by Dr. Y. Hirai, Immunological

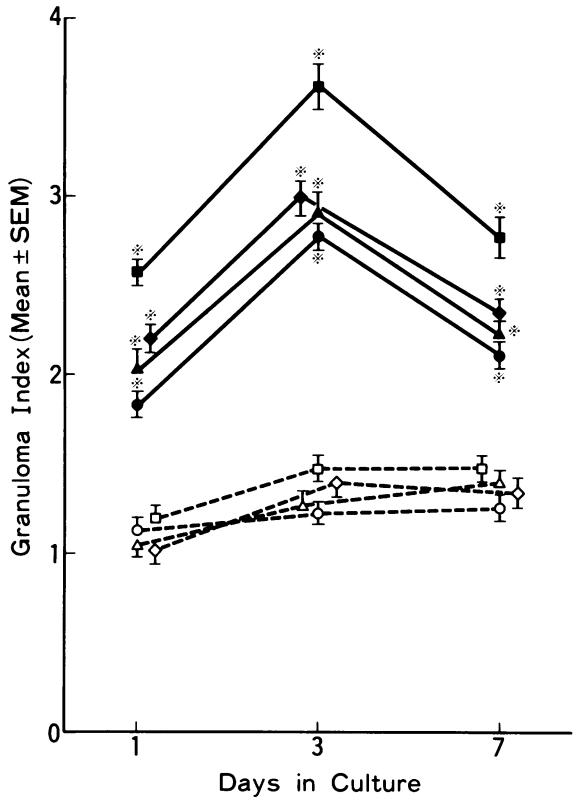

Figure 3. *Kinetics of* in vitro *granuloma formation. Balb/c mouse spleen cells were cultured in the presence of dextran (●) or latex beads (○). Spleen cells from C57BL/6 mice were cultured in the presence of dextran (▲) or latex beads (△). C3H/He mouse spleen cells were cultured in the presence of dextran (■) or latex beads (□). Spleen cells from athymic nude mice were cultured in the presence of dextran (◆) or latex beads (◇). Data represent the mean GI ± SEM from eight independent experiments of three mice per each condition. Statistic differences (*) relative to cellular reactions induced by latex beads (P < 0.02).*

Products and Development, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan.[14] Recombinant human TNF-α with a specific activity of 2×10^7 U/mg was supplied by Asahi Chemical Industry, Co., Ltd., Tokyo, Japan.[15] Recombinant human IL-2 (specific activity, 1×10^7 U/mg) and recombinant murine IFN-γ (specific activity, 1×10^7 U/mg) were gifts from Shionogi Pharmaceutical Co., Ltd., Osaka, Japan.[16,17] Recombinant cytokines were coupled to agarose beads (Pharmacia Fine Chemicals), and the residual active groups were blocked by 1 M of ethanolamine (Wako Chemical, Tokyo, Japan) as described previously.[7,10,18] Similarly, agarose beads coupled to unrelated protein, bovine serum albumin (BSA; Sigma, St. Louis, MO), and plain beads were activated, ethanolamine-plugged, and formaldehyde sterilized. Approximately 70 to 80% of cytokine activities were bound to the beads examined by bioassays. Thus, 100 to 300 U of cytokine activity (IL-1, TNF-α, IL-2, or IFN-γ) were bound to 3×10^3 agarose beads.

Bioassays for Cytokine Activities

Cell-free culture supernatants were collected at various intervals after incubation and were assayed for cytokine activities. The thymocyte proliferation activity of IL-1 was determined by its capacity to stimulate Balb/c mouse thymocytes in the presence of phytohemagglutinin (PHA; Difco Laboratories, Detroit, MI), as described.[19] IL-2 activity was assayed by its ability to stimulate proliferation of the IL-2-dependent cell line (CTLL-2).[20] The CTLL-2 was provided by Dr. Kendall A. Smith. Proliferation was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma) colorimetric assay.[21] In all assays, a unit of activity was defined as the amount of materials per milliliter producing a half maximal response.

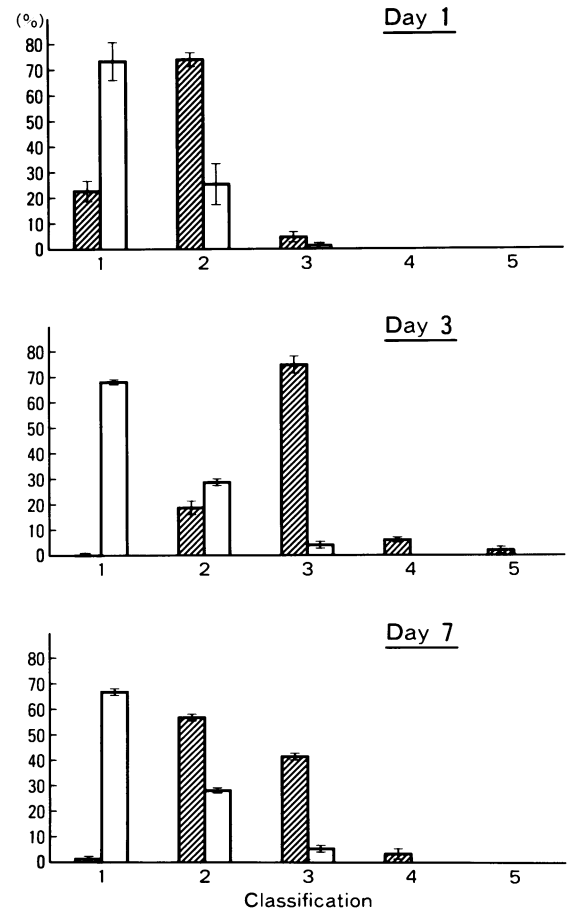

Figure 4. *Morphologic classification of* in vitro *granuloma formation after 1, 3, and 7 days of culture. The histograms illustrate the distribution of reactions expressed as a percentage of the total for category at 1, 3, and 7 days of culture. The hatched bars (▨) represent the reactions of Balb/c mouse spleen cells to dextran beads, and open bars (□) represent the reactions of cells to latex beads. The distributions shown here are representative of data from six experiments.*

Data Analysis

Statistical analysis of data with respect to controls was performed using the Student's *t*-test; *P* values less than 0.05 were considered significant.

Results

Demonstration of Granulomas In Vitro

Experiments were first performed to assess the ability of spleen cell cultures to respond to artificial microparticles *in vitro*. *In vitro* granulomas were developed when spleen cells from Balb/c (H-2^d), C57BL/6 (H-2^b), C3H/He (H-2^k) or athymic nude (H-2^d) mice were cultured with dextran beads (Figure 1A–D). Granulomas induced by dextran beads were conspicuous by day 1, reached maximum size (GI, 2.7 to 3.5) by day 3, and then gradually decreased in size thereafter. The lesions were composed predominantly of macrophages with fewer neutrophils. No proliferation of cells was observed in granuloma-generating cultures using by MTT colorimetric assay. In contrast, the inflammatory reactions induced by latex beads were considerably smaller and were composed of macrophages. Scanning electron micrographs of day 3 granulomas induced by dextran beads showed numerous pseudopodia extending from the surface of macrophages into the dextran bead material and macrophages bound tightly the beads (Figure 2A, B). Spleen cells from all strains of mice examined in this study produced granulomas *in vitro* when cells were cultured with dextran beads but not with latex beads. A summary of the time course of murine granuloma formation *in vitro* of each strain is shown in Figure 3. A more precise evaluation of these reactions is shown in Figure 4. The data are expressed in percentages of cellular responses in each classification. After 3 days of culture, there was a significant increase in the number of cellular reactions to dextran beads but not to latex beads. By day 7, the responses decreased gradually. The response to artificial microparticles (dextran and latex beads), histologic characteristics, and kinetics of granulomas *in vitro* shown in the present study correlated well with granuloma formation *in vivo*.[7,8,10]

Requirement of Macrophages for Induction of Granulomas In Vitro

In view of the predominant participation of macrophages in granulomas *in vivo*[7,8,10] and *in vitro*, as described above, we examined cellular requirements for induction

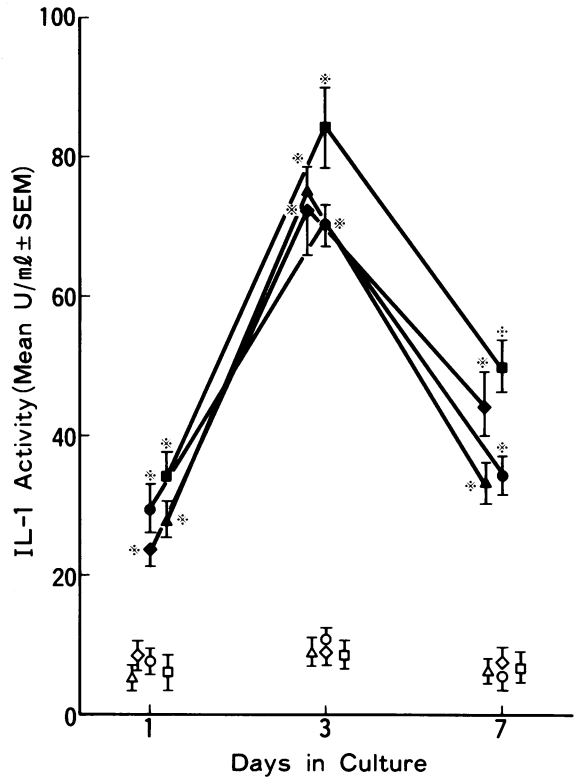

Figure 5. *IL-1 activity in culture supernatants of spleen cell granulomas* in vitro. *Balb/c mouse spleen cells were cultured in the presence of dextran (●) or latex beads (○). Spleen cells from C57BL/6 mice were cultured in the presence of dextran (▲) or latex beads (△). C3H/He mouse spleen cells were cultured in the presence of dextran (■) or latex beads (□). Spleen cells from athymic nude mice were cultured in the presence of dextran (◆) or latex beads (◇). Data represent the mean IL-1 activity (U/ml) ± SEM from eight independent experiments of three mice per each condition. Statistic differences (*) relative to IL-1 activities induced by latex beads (P < 0.02).*

of the lesions. Adherent cells from either Balb/c or athymic nude mice were necessary for induction of granuloma formation by dextran beads. Dextran bead-induced granulomas with adherent cells were prominent by day 1, reached peak intensity (GI, 2.7 to 3.0) by day 3, and gradually declined in size thereafter. The histologic fea-

Table 1. *Lack of CTLL Proliferation Activity in Granuloma Culture Supernatants**

Samples from	CTLL proliferation activity (U/ml)		
	Day 1	Day 3	Day 7
Balb/c	<0.2	<0.2	<0.2
C57BL/6	<0.2	<0.2	<0.2
C3H/He	<0.2	<0.2	<0.2
nu/nu	<0.2	<0.2	<0.2

* Data represent the mean CTLL proliferation activity (U/ml) ± SEM of triplicate samples from five independent experiments. Murine spleen cells were cultured in the presence of dextran beads. Standard error of the mean was less than 5%. IL-2 and IL-4 activities were assayed by using CTLL cells. Recombinant human IL-2 and IL-4 (100 U/ml) were employed as positive controls of the assay.

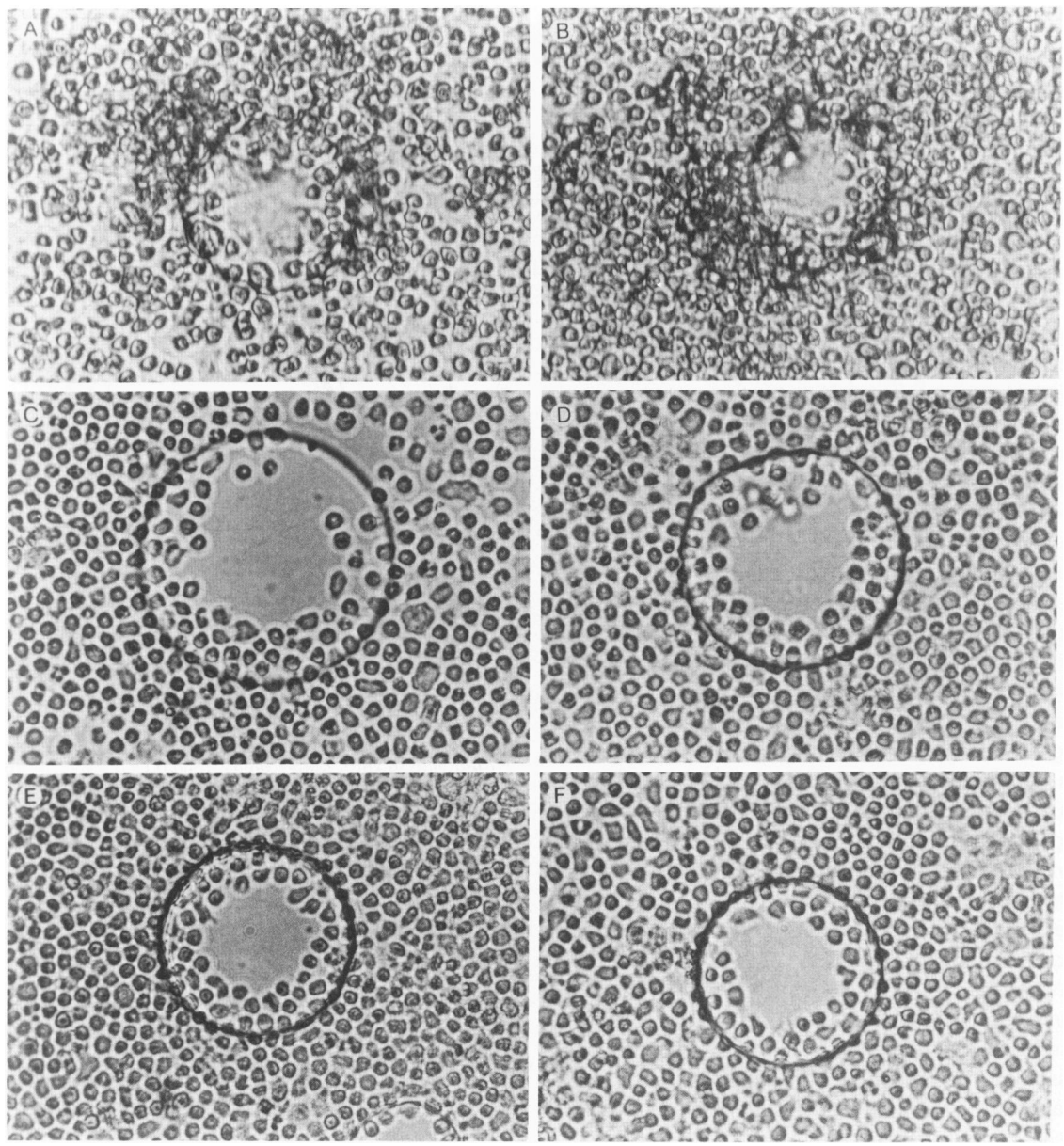

Figure 6. *Granuloma formation* in vitro *by recombinant cytokine-coupled beads.* **A, B:** *Representative lesions seen in 3 days after culture of Balb/c mouse spleen cells in the presence of agarose beads coupled to either IL-1 (A) or TNF-α (B). Large granulomas are observed, and the cellular components are mainly macrophages (×150).* **C, D:** *Cellular reactions seen 3 days after culture of spleen cells in the presence of agarose beads coupled to either IL-2 (C) or IFN-γ (D).* **E, F:** *Cellular reactions seen in 3 days after culture of spleen cells in the presence of BSA-coupled (E) or plain agarose beads (F). The beads (C to F) are surrounded by a mild infiltrate of macrophages but are not attached to the bead surface.*

tures and time-kinetics of the granulomas were similar to those induced by whole spleen cells (data not shown). Adherent cell populations consisted of more than 95% macrophages examined by nonspecific esterase staining, suggesting that macrophages are responsible for induction of granulomas *in vitro*. In contrast, neither nonadherent cells nor thymocytes could induce granulomas *in vitro*.

Cytokine Activity in Granuloma Culture Supernatants

We also examined cytokine activity in the culture supernatants, because it has been reported that cytokines play an important role in induction of murine granulomas *in vivo*.[7,9,10,22–24] IL-1 activity in granuloma supernatants of spleen cell cultures in the presence of dextran beads was

detectable within 1 day and reached peak activity by 3 days after the culture. A decrease in IL-1 activity was observed 7 days after the culture (Figure 5); however, very little IL-1 activity was found in the supernatants of spleen cell cultures in the presence of latex beads. The temporal profile of IL-1 activity in the supernatants was almost identical to the profile of granuloma development *in vitro* (Figure 3). Granuloma-derived IL-1 activity was observed in coculture supernatants of dextran beads and spleen cells from all strains of mice used in this study.

Thymocyte proliferation assay with suboptimal concentration of PHA reflects several interleukin activities (IL-1, IL-2, and IL-4) in the samples[25-27] and CTLL cells could respond to IL-4[27] as well as IL-2.[26] We therefore examined IL-2 and IL-4 activities using CTLL proliferation assay to clarify the nature of thymocyte proliferative response in the standard IL-1 assay described above. As shown in Table 1, no CTLL proliferation activity was detected in the granuloma culture supernatants that had thymocyte proliferative activity. Thus, neither IL-2 nor IL-4 activity was detected in the supernatants. The result that no IL-2 or IL-4 activity was detected by bioassay could not preclude the possibility of presence of antigenic materials in the samples. Further examination of lymphokines using an immunochemical method such as Western blot is required to confirm the results.

Our failure to detect both IL-2 and IL-4 could be due either to a relative inability of granuloma cells to produce these lymphokines or the presence of inhibitory factors that interfere with the expression of IL-2 and/or IL-4 activity. We therefore examined the ability of the supernatants to neutralize or inhibit the proliferative activity of recombinant IL-2 and IL-4. No significant neutralizing activity against IL-2 and IL-4 was detected in the supernatants (data not shown). The results suggest that the activity was in fact due to IL-1. Furthermore, IL-1 activity was found in culture supernatants of dextran beads with adherent cells (macrophages) but not with nonadherent cells (data not shown). The kinetics was similar to that of *in vitro* granulomas induced in the adherent cell culture in the presence of dextran beads. Again, these supernatants did not contain any detectable IL-2 and IL-4 activity measured by CTLL cell proliferation assay (data not shown).

Induction of Granulomas In Vitro *by Recombinant Monokine-Coupled Beads*

The result that macrophages and their mediators are involved in granuloma formation *in vitro* prompted us to examine the possibility of induction of granulomas by recombinant cytokine-coupled beads. Because cytokines (IL-1, TNF-α, and IL-2) are active across species

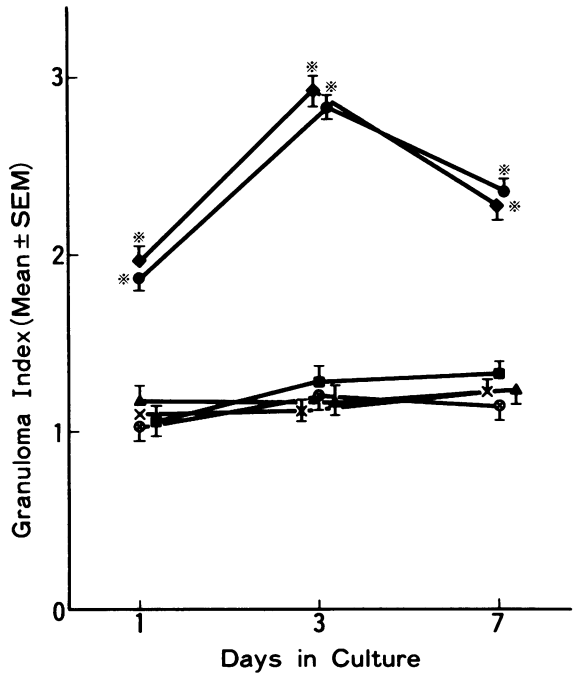

Figure 7. *Kinetics of granuloma formation* in vitro *by cytokine-coupled agarose beads. Spleen cells from Balb/c mice were cultured in the presence of recombinant IL-1-coupled (●), recombinant TNF-α-coupled (♦), recombinant IL-2-coupled (▲), recombinant IFN-γ-coupled (■), BSA-coupled (⊗), or plain agarose beads (×). Data represent the mean GI ± SEM from four independent experiments of three mice per each condition. Significant difference (*) when compared with lesions induced by IL-2-coupled, IFN-γ-coupled, BSA-coupled, or plain beads (P < 0.02).*

line,[25,26,28] we used recombinant IL-1, TNF-α, and IL-2 of human origin. Because the activity of IFN-γ is restricted by species,[29] we used recombinant murine IFN-γ in this experiment. As shown in Figure 6A and B, granulomas were able to induce granulomas by agarose beads coupled to monokines such as IL-1 and TNF-α. *In vitro* granulomas generated by spleen cells in the presence of recombinant IL-1 or TNF-α coupled agarose beads were prominent by day 1, reached peak intensity (GI, 2.7 to 3.0) by day 3, and gradually declined in size thereafter. A summary of the time-course of *in vitro* granuloma formation by spleen cells in the presence of cytokine-coupled beads is shown in Figure 7. The histologic features and time-kinetics of the granulomas were similar to dextran bead-induced lesions (Figures 1 to 3). Also, the distribution of agarose beads coupled to granulomatogenic cytokines, including IL-1 and TNF-α with various degrees of cellular reactions, was similar to that of dextran beads (Figure 4). Beads coupled to T lymphokines such as IL-2 and IFN-γ were unable to induce granulomas (Figure 6C, D). No significant granulomas *in vitro* were generated in the presence of plain agarose beads or BSA-coupled beads (Figure 6E, F). It is consistent with previous results

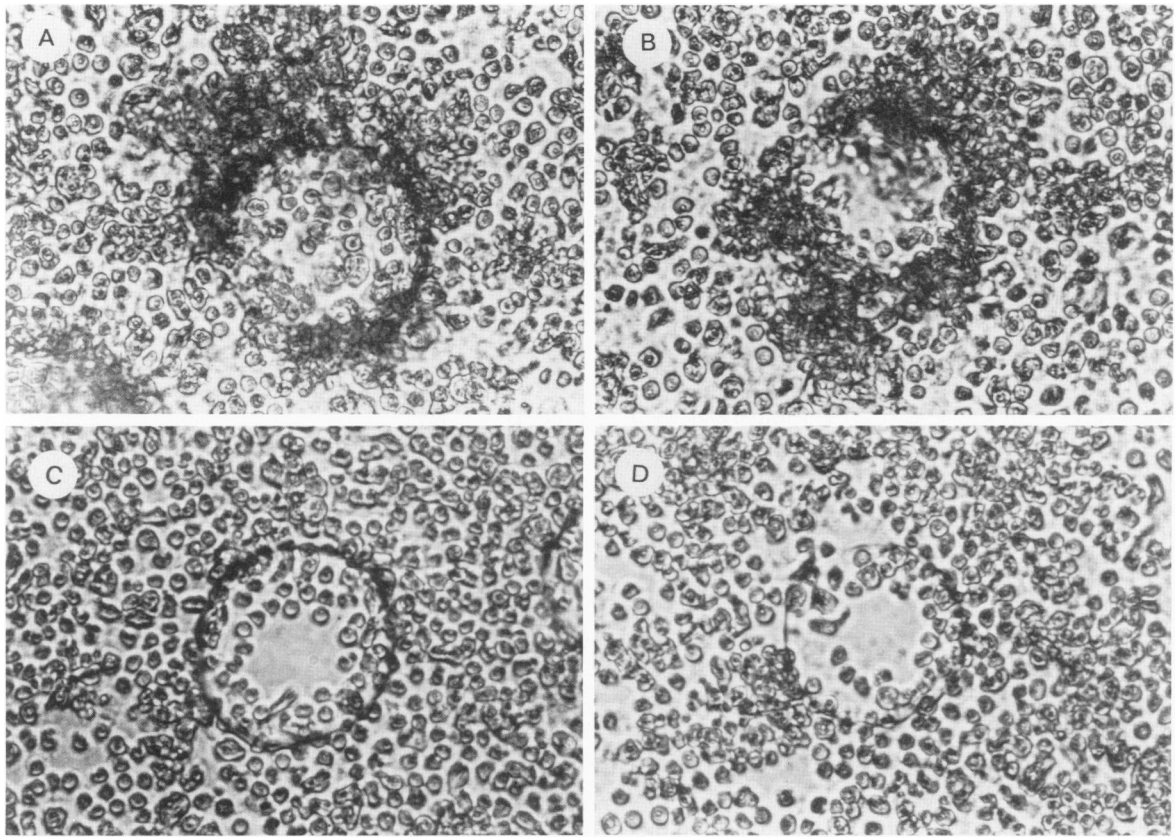

Figure 8. *Granuloma formation* in vitro *by adherent macrophages and monokine-coupled beads.* **A, B:** *Representative lesions seen 3 days after the culture of adherent macrophages in the presence of agarose beads coupled to either IL-1 (A) or TNF-α (B). Large granulomas are observed (×150).* **C, D:** *Cellular reactions seen 3 days after culture of adherent cells in the presence of agarose beads coupled to either IL-2 (C) or IFN-γ (D). The beads (C and D) are surrounded by a very mild infiltrate of cells (×150).*

using *in vivo* systems that agarose beads themselves are inert.[7,9,10] The kinetics and histology of the lesions were similar to those induced by beads coupled to T lymphokines (Figures 6C–D and 7). The distribution of cellular reactions by plain agarose beads or beads coupled to unrelated protein (BSA) or T lymphokines such as IL-2 and IFN-γ was analogous to that of latex beads. Thus, it is unlikely that IL-2 and IFN-γ are inhibiting macrophage responsiveness. These results show that T lymphokines such as IL-2 and IFN-γ had little effect on both induction and suppression of granulomas and agarose beads themselves had no granulomatogenic activity. We used the cytokine concentration (100 to 300 U/3 × 10^3 beads in culture) that was equivalent to activity produced *in vitro* by the dextran system. We performed experiments by increasing concentration of cytokines on beads. The results showed that prominent granulomas were seen in the presence of beads coupled to monokines but not T lymphokines. Large granulomas were observed when adherent macrophages were cultured in the presence of agarose beads coupled to either IL-1 or TNF-α (Figure 8A, B). The histology and kinetics of the lesions produced by culturing macrophages in the presence of monokine-coupled beads were similar to those of the lesions induced by culturing spleen cells with dextran beads or monokine-coupled beads. Minimal lesions were induced in the cultures of adherent macrophages with plain beads or beads coupled to IL-2 or IFN-γ (Figure 8C, D). Also, very mild reactions were seen in the culture of nonadherent cells with beads coupled to monokines or T lymphokines. A summary of the time-course of *in vitro* granuloma formation by macrophages and cytokine-coupled beads is shown in Figure 9.

Discussion

The present study demonstrated that granulomas can be generated in *in vitro* systems by culturing spleen cells from various strain of mice with artificial microparticles. Generally, granuloma-inducing agents persist in the tissues because they are insoluble or poorly degradable.[1,2] In these experiments, we used dextran or agarose beads as the nidi for the lesions. We have reported previously

that dextran-induced granulomas in mice are foreign-body type lesions, because the lesion was inducible in normal, athymic nude, and immunologic dextran-nonresponder (CBA/N) mice. Additionally, the granuloma response of mice to dextran beads was not enhanced by pre-immunization with the beads.[8] The histologic features and kinetics of *in vitro* granulomas were consistent with *in vivo* lesions.[7,8,10] The culture supernatants contained monokine activity in association with granuloma formation. Furthermore, agarose beads coupled to recombinant monokines such as IL-1 and TNF-α, but not T cell-derived lymphokines (IL-2 and IFN-γ), were able to induce granulomas *in vitro*. Macrophages were necessary for the development of *in vitro* granulomas by dextran beads and by monokine-coupled beads. These results suggest that macrophages and monokines such as IL-1 and TNF-α play an essential role in the development of granulomas.

Granuloma formation is the expression of a series of complex inflammatory events. In recent years, much attention has been focused on the role of cytokines in the initiation and maintenance of murine granuloma formation *in vivo*.[7-10,22,23] Also, it has been reported that cytokines may participate in the cascade of events leading to granuloma formation of human sarcoidosis.[30-35] In both hypersensitivity and foreign-body pulmonary granuloma models in mice, IL-1, but not IL-2, was detected in the lesions in association with activity and size of granulomas.[7,9,10] We have reported that IL-1 can initiate the induction of granulomas in mice.[10] Taken together with the results of *in vitro* studies described in this paper, macrophages and monokines participate in the basic mechanisms of granulomatous inflammation. Histopathologically, the bulk of granulomas both *in vivo* and *in vitro* is composed of macrophages and their derivatives. The lesions induced by macrophage-enriched populations showed similar kinetics of granuloma formation by whole spleen cells. Thus, macrophages and monokines were required for both development and regulation of granulomatous lesions. In addition, the time-kinetics of *in vitro* granulomas appears to correlate well with that of *in vivo* lesions.[7-10] These results suggest that the *in vitro* granuloma model is a powerful tool for investigating the basic mechanism of development and regulation of granulomatous diseases.

Macrophages and monocytes secrete a wide range of biologically active mediators in response to inflammatory stimuli.[36] Some of these products, such as IL-1 and TNF-α, are well known, although the role of monokines in the pathogenesis of granulomatous inflammation remains unknown. Monokines have multiple effects on cells involved in inflammation.[3-5,28,36] Furthermore, IL-1 and TNF-α share multiple biologic activities. The combined effects of these two distinct monokines is often greater than either one

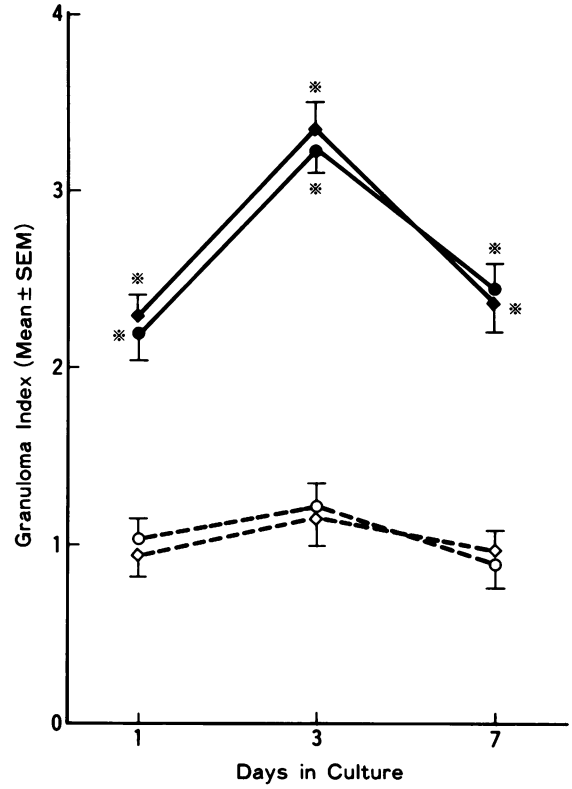

Figure 9. *Kinetics of granuloma formation* in vitro *by macrophages with monokine-coupled agarose beads. Splenic macrophages from Balb/c mice were cultured in the presence of beads coupled to recombinant IL-1 (●) or recombinant TNF-α (♦). Adherent cells were cultured in the presence of beads coupled to IL-2 (○) or IFN-γ (◇). Data represent the mean GI ± SEM from three independent experiments of three mice per each condition. Statistic difference (*) relative to lesions induced by adherent cells in the presence of T lymphokine-coupled beads (P < 0.02).*

alone. This synergism of IL-1 and TNF-α has been observed in inflammatory responses.[3,4,28] Monokines released from activated monocytes and macrophages in turn stimulate monocytes and macrophages. Such a mechanism of cell–monokine interaction provides for a powerful amplification circuit in the initial phase of the inflammatory response.[3-5,25] In view of the predominant participation of macrophages in experimental granulomas *in vivo*[7-10] and *in vitro*, it is reasonable to assume that chemotactic activity and inflammatory signals of monokines[3-5,25,28] are responsible for the initiation and/or development of granulomas. Thus, formation of granulomas requires interactions between macrophages and monokines.

Alternatively, T lymphocytes and T cell-derived lymphokines including IL-2 and IFN-γ could not produce experimental granulomas *in vitro*. Taken together with our previous results that lack of IL-2 activity in murine granulomas[7,9,10] and *in vivo* lesions that we were able to induce in athymic nude mice and in dextran nonresponder mice

(CBA/N),[8] these data may prove that T lymphocytes and T lymphokines have a small role in foreign-body granuloma formation both *in vivo*[8,10] and *in vitro,* as described in this paper. Certain T lymphokines, including migration inhibition factor and macrophage chemotactic factor as well as monokines, play important roles in granuloma formation, however, especially in hypersensitive-type lesions.[7,9,12,22-24] Further study is necessary to confirm the possibility.

The results described in this paper provide novel evidence for the ability of macrophages and monokines (including IL-1 and TNF-α, but not T-lymphokines) to induce murine granulomas *in vitro.* Thus, macrophages and monokines play an essential role in the development of granulomatous inflammation. This model of granulomatous inflammation *in vitro* may be a useful tool for investigating not only the mechanisms of granuloma formation but also the role of cytokines.

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