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INVESTIGATIVE REPORT

Langhans-type and Foreign-body-type Multinucleated Giant Cells in Cutaneous Lesions of Sarcoidosis

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Multinucleated giant cells are characteristic of a monocyte–macrophage lineage in sarcoidosis and consist of two types of cells: Langhans-type with an arcuate arrangement of nuclei and a foreign-body type with random arrangement of nuclei. To compare these cells in the cutaneous lesions of sarcoidosis, we histologically and immunohistologically examined multinucleated giant cells in 25 scar infiltrations (cutaneous sarcoidosis with foreign bodies) and 30 cutaneous lesions of sarcoidosis without foreign bodies. Regardless of the presence or absence of foreign bodies, the cutaneous lesions had both types of multinucleated giant cells, usually with a predominance of the Langhans-type, although the numbers of total multinucleated giant cells were higher in scar infiltrations than in cutaneous sarcoidosis without foreign bodies, suggesting that their frequency is influenced by the microenvironment in sarcoidal lesions such as the presence of foreign bodies. Immunohistochemical studies using surface antigens of monocyte–macrophage lineage cells and adhesion molecules indicated that both types of multinucleated giant cells are formed from monocytes rather than tissue macrophages and are phenotypically the same cells with different distributions of nuclei. *Key words: immunohistochemical staining monocyte; scar infiltrations.*

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Sarcoidosis is expressed as various types of organ involvement including cutaneous lesions. Cutaneous involvement can be either specific skin lesions with sarcoidal granulomas or non-specific skin lesions that are mainly reaction patterns, such as erythema nodosum (1–3). The specific skin lesions include nodules, plaques, lupus pernio, subcutaneous lesions or, occasionally, lichenoid lesions (4), psoriasis-like and erythema nodosum-like lesions (5). The most common histological features of these specific lesions are epithelioid cell granulomas, which histologically consist of epithelioid cells, multinucleated giant cells (MGCs) and, infrequently, T lymphocytes. Scar infiltration is another cutaneous lesion of sarcoidosis in which foreign bodies, mainly silica, are present. This type is considered to be histologically different from specific cutaneous lesions of sarcoidosis.

MGCs are classified as Langhans-type (LGC) or foreign-body-type (FGC) cells based on their nucleus array: LGC are characterized by the arcuate arrangement of their nuclei, while FGC have randomly arranged nuclei. LGCs are considered to be characteristic of sarcoidosis and tuberculosis, while FGCs have been reported to be predominant in foreign-body granulomas (6). However, it is not known whether there is any phenotypical or functional difference between the two types of MGCs. Van der Rhee et al. (7) reported that LGCs may be precursors of FGCs based on the number of nuclei. However, both types of MGCs are simultaneously produced from peripheral blood monocytes treated with supernatants of concanavalin A-stimulated mononuclear cells (8), suggesting that the MGCs are identical except for a different arrangement of their nuclei. Here we examine the phenotypic profiles of both types of MGCs and the frequency with which they occur in sarcoidal lesions with or without foreign bodies.

MATERIAL AND METHODS

Patients

The 55 subjects analyzed in this study included patients diagnosed between 1985 and 1997 as having sarcoidosis with cutaneous lesions. Twenty patients had nodular lesions, 10 had plaque lesions and 25 had scar infiltration lesions all located on the knees. The nodular type of sarcoidosis is characterized by yellowish-brown or red papules 5–15 mm in diameter, and the plaque type shows an annular configuration. Scar infiltration lesions develop in traumatic scars and the areas of predilection are the knees, elbows and face. The clinical features of scar lesions are erythematous papules, nodules or plaques. Sometimes the lesions are arranged linearly. In our study, some lesions were not noticed by the patients because they were small and asymptomatic. The scar infiltration-type lesion is histologically diagnosed by polarization microscopy for foreign-body material. Some of the patients came to our department because of skin lesions, and others were referred to us from other departments or hospitals to confirm the diagnosis of sarcoidosis. The patients with tiny or asymptomatic lesions belong to the latter group.

All patients were examined in the Departments of Dermatology, Internal Medicine and Ophthalmology. The onset of the lesions and their association with activity of other organ involvement in the sarcoidosis were not determined when the skin lesions were small. Infectious conditions that were

specially stained for microorganisms were excluded. Kveim reagin is not available in Japan, and testing was not performed. A diagnosis of sarcoidosis was based on the presence of more than one additional extracutaneous clinical feature of sarcoidosis such as uveitis, lymph node swelling, intra-thoracal lesions such as bilateral hilar lymphadenopathy shown on chest X-ray or CT examination, and respiratory symptoms such as cough, and more than one characteristic laboratory abnormality of the disease, including purified protein derivative reaction anergy, high serum levels of γ-globulin, angiotensin converting enzyme, or lysozyme and accumulation of gallium scintigraphy, plus the histological evidence of sarcoidal granulomas. Other systemic disorders such as malignant lymphoma, tuberculosis and berylliosis, and local granuloma reactions caused by foreign bodies and malignant tumor were excluded before a diagnosis of sarcoidosis was made.

Methods

Tissue preparation. A 3-mm punch biopsy was taken, fixed in 10% formaldehyde and embedded in paraffin for hematoxylin-eosin staining to enumerate the number of MGCs and their nuclei. Additional biopsies taken from 10 patients were snap frozen in liquid nitrogen and stored at $-80°C$ until processing for the immunohistochemical examinations.

Enumeration of the number of MGCs and their nuclei. MGC contains > 3 nuclei/cell. The number of LGCs, FGCs and the total number of MGCs were counted in the whole area of one section that was randomly selected from $6-8$ serial sections of a 3-mm punch biopsy. The total number of nuclei in each MGC was also counted. Statistical significance of differences was determined by Student's *t*-test and a *p*-value of less than 0.05 was considered to be significant.

Immunohistochemical staining of MGCs. Cryostat sections were cut at a thickness of 4 μm and fixed in acetone for 30 min at 4°C after drying at room temperature. Cell surface antigens were detected using the following monoclonal antibodies: anti-CD11a, anti-CD11b (Pharmingen, San Diego, USA), anti-CD9, anti-CD13, anti-CD14, anti-CD15, anti-CD16, anti-CD68, MAC387, 3A5 (Novocastra Lab, Newcastle, UK), anti-CD1a, anti-CD11c, anti-CD36, anti-CD45RO, anti-CD54, anti-CD49d, anti-CD62, anti-CD86 and anti-CD106 (Serotec, Oxford, UK). A specific monoclonal antibody for MGL, macrophage Gal/Gal NAc-specific calcium-type lectin, was a gift from Dr Irimura (9). The sections were post-fixed in 4% paraformaldehyde for 5 min and endogenous peroxidase activity depleted with 3% hydrogen peroxide for 20 min. The sections were incubated with the above primary antibodies for 60 min and then with biotinylated goat anti-mouse antibody for 30 min, followed by an avidin-biotinylated-peroxidase reagent for 45 min (Vector Laboratories, Peterborough, UK). Peroxidase activity was disclosed with 0.5 mg/ml 3,3′-diaminobenzidine (Sigma) using 0.3% hydrogen peroxide as the substrate.

RESULTS

Fifty-two out of 55 cutaneous lesions of sarcoidosis patients examined had MGCs. Forty lesions had both types of MGCs (Fig. 1) and 12 had only LGCs. There were no lesions having only FGCs. Three lesions without MGCs were from an acute form of sarcoidosis,
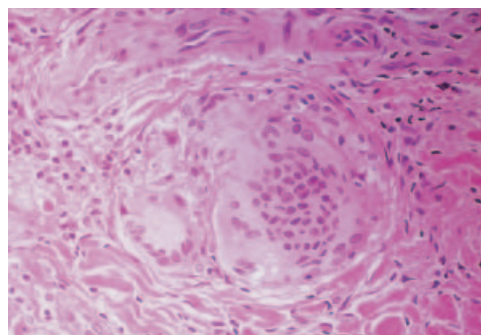
Fig. 1. Langhans-type (*left*) and foreign-body type (*right*) multinucleated giant cells in cutaneous biopsy from nodular sarcoidal lesion (Hematoxylin & Eosin stain, $\times 400$).

in which the biopsy samples were taken from the early cutaneous lesions. Two lesions had more than 40 MGCs in a biopsy section, in which lymphocytes were occasionally observed around the granulomas. One patient had had skin lesions for more than 5 years. The second patient noticed the lesions one year before undergoing a skin biopsy.

Table I shows that there was a significant difference in the frequency of both the total and of each type of MGC between lesions with and without foreign bodies. The average number of nuclei in MGCs was 10.1 ± 6.5. This figure was significantly different between LGC and FGC cells, which had 7.9 ± 4.1 and 14.2 ± 7.9 nuclei, respectively ($p < 0.05$). There were no differences in the number of nuclei in each MGC between scar infiltrates and cutaneous sarcoidosis without foreign bodies. In scar infiltrates, foreign bodies were seen not only in FGCs but also in LGCs. Immunohistological studies showed that both types of MGCs had the same profile of macrophage–monocyte lineage cell surface markers and adhesion molecules. Anti-CD54 mAb, anti-CD9 mAb, anti-CD13 mAb and anti-CD14 mAb

Table I. *The frequency and types of multinucleated giant cells (MGCs) in scar infiltrates and sarcoidal cutaneous lesions without foreign bodies*

Types of MGC	Scar infiltrates	Cutaneous lesions without foreign bodies	All cutaneous lesions
LGC, *n* (%)[a]	11.5 ± 8.2*[#] (63.5%)[a]	6.2 ± 4.6** (73.8%)	8.6 ± 7.0*** (67.2%)
FGC, *n* (%)	6.6 ± 5.7[#] (36.5%)	2.2 ± 2.7 (26.2%)	4.2 ± 4.8 (32.8%)
Both types, *n*	18.1 ± 12.8[#]	8.4 ± 7.0	12.8 ± 11.1

[a]Percentage of number of each MGC/number of total MGC in a skin lesion group.
*$p < 0.02$ vs. FGC in scar infiltrates.
**$p < 0.001$ vs. FGC in cutaneous lesions without foreign bodies.
***$p < 0.01$ vs. FGC in all cutaneous lesions.
[#]$p < 0.001$ vs. each type of MGC in cutaneous lesions without foreign bodies.
LGC: Langhans-type cells; FGC: foreign-body type cells.

reacted with MGCs as well as epithelioid cells (Fig. 2). On the other hand, 3A5, a macrophage marker mAb obtained by immunization of mice with human spleen cell homogenate (10), reacted with cytoplasmic areas of epithelioid cells and MGCs (Fig. 3). They also stained with a macrophage Gal/Gal NAc antibody (Fig. 4). CD11a and CD68 were weakly expressed in MGCs. Both types of MGC and epithelioid cells in granulomas were negative for CD1a, CD15, CD16, CD36, CD45RO, CD49d, CD62L, CD68, CD86, CD106, MAC387 and MGL.

DISCUSSION

A characteristic pair of infiltrating cells in sarcoidal granulomas is monocyte–macrophage lineage cells and their fused cells, LGC and FGC (11). In the current study we showed that both types of MGCs were present but with a predominance of LGCs in sarcoidal cutaneous lesions both with and without foreign bodies. The surface markers for monocyte–macrophage lineage cells were the same in both MGC types, suggesting that LGC and FGC cells are derived from the same stem cells. However, it is not known why LGCs are predominantly present in sarcoidal lesions. Van der Rhee et al. (12) reported that the morphology of MGCs is closely related to the number of nuclei present in foreign-body granulomas induced by subcutaneous implantation of pieces of Melinex plastic: LGCs generally had less than 10 nuclei and FGCs generally contained more than 30 nuclei. In our study, the number of nuclei in FGCs (14.2 ± 7.9) was significantly higher than that in LGCs (7.9 ± 4.1), which supports van der Rhee et al.'s findings.

A second explanation for the predominance of LGC cells is that each type of MGC is determined by the cytokines produced in sarcoidal lesions. Both types of MGCs are experimentally produced by treatment of peripheral blood monocytes with supernatants of concanavalin A-stimulated mononuclear cells and cytokines (7, 13–19). Using this type of *in vitro* system, we recently reported (19) that the ability of monocytes to
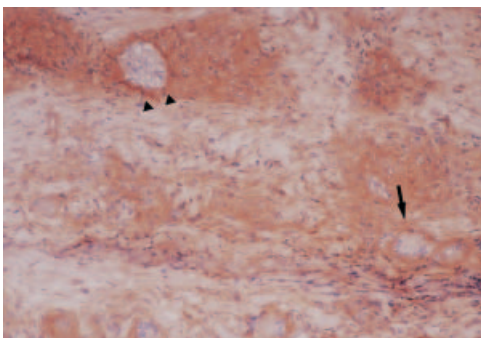

Fig. 2. Immunostaining of CD54 in Langhans-type (*arrow*) and foreign-body type (*arrow heads*) multinucleated giant cells.
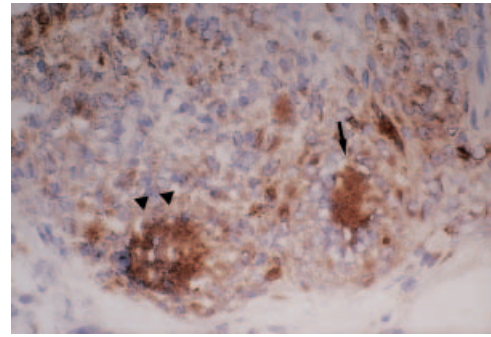

Fig. 3. Immunostaining of 3A5 in Langhans-type (*arrow*) and foreign-body type (*arrow heads*) multinucleated giant cells.
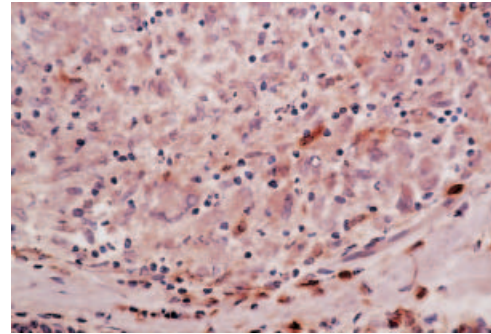

Fig. 4. Immunostaining of a macrophage Gal/Gal NAc-specific calcium-type lectin in a sarcoidal lesion.

form MGCs was enhanced through P2X7 receptors in sarcoidosis patients. McNally & Anderson (15) reported that IFN-γ preferentially induced LGC, while IL-4 led to FGC formation. On the other hand, our previous studies showed that IFN-γ was associated with the generation of both types of MGCs and that GM-CSF and IL-3 were implicated in LGC formation (18). In sarcoidal granulomas, several kinds of cytokines are produced from lymphocytes and monocyte–macrophage lineage cells (20).

A third factor is the relevance of adhesion molecules of monocyte–macrophage lineage cells. In sarcoidal lesions, Shimizu et al. (21) reported that the epithelioid cells in lung lesions of sarcoidosis were ICAM-1^{+}, ECAM-1^{-} and VCAM-1^{-}. Most et al. (22) reported that expression of LFA-1 on monocytes was necessary for MGC formation. On the other hand, the change in ICAM-1 expression and cellular distribution has been emphasized to be important in the mechanism of IFN-γ-induced MGC formation (23). Our immunohistological studies showed that epithelioid cells as well as both types of MGC in cutaneous lesions of sarcoidosis had a high expression of ICAM-1, while LFA-1 was highly expressed in epithelioid cells but weakly expressed in both types of MGCs. This suggests that adhesion molecules are necessary for MGC formation but do not determine the type of MGC.

A fourth factor is the association of pathogens or

insoluble materials present in sarcoidal lesions. We previously found that muramyl dipeptide (MDP), a synthetic adjuvant, induced LGC from monocytes stimulated by supernatants of concanavalin A-stimulated mononuclear cells (18). MDP is one of the bacterial peptidoglycans, such as mycobacteria and *Propionibacterium acnes*, which have been suggested to be causative agents of sarcoidosis (24). Furthermore, since silica, a common foreign body present in scar infiltration of cutaneous sarcoidosis, has been proved to possess adjuvant effects (25), such an unsoluble body may affect monocytes and enhance their fusion rate.

MGCs formed *in vitro* probably originate from monocytes themselves rather than macrophages, because Most et al. (8) reported that cells differentiated to macrophages during long-term culture. We earlier reported (18) that $CD14^+/CD16^-$ monocytes but not $CD14^+/CD16^+$ monocytes were fused to form MGC. The current study showed that both types of MGCs reacted with anti−monocyte/macrophage markers such as CD14, 11a and 3A5, but not with anti-macrophage markers such as CD16 and MAC387. MGL, a specific marker for tissue macrophages (9), was also negatively stained in both MGCs and epithelioid cells. These results support the idea that MGCs in sarcoidal granulomas are derived from monocytes but not from tissue macrophages.

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