Monocyte-derived multinucleated giant cells and sarcoidosis

Hiroyuki Okamoto*, Kana Mizuno, Takeshi Horio

Department of Dermatology, Kansai Medical University, 10-15 Fumizono, Moriguchi, Osaka 570-8507, Japan

Received 25 September 2002; received in revised form 24 October 2002; accepted 28 October 2002

KEYWORDS
Granuloma;
Monocyte;
Muramyl dipeptide;
Purinergic receptors;
Cytokines

Summary Multinucleated giant cells (MGC) are characteristic cells in granulomatous disorders such as sarcoidosis and also formed in vitro from peripheral blood mononuclear cells by stimulation with cytokines, including interferon-γ (IFN-γ), interleukin-3 (IL-3), IL-4, IL-13, and granulocyte-macrophage-colony stimulating factor. In addition to such inflammatory mediators, a factor derived from the pathogens of granulomatous disorders may be necessary for MGC formation. Muramyl dipeptide (MDP), a peptidoglycan portion of bacterial cell walls present in sarcoidal lesions, is one of the candidates and can preferentially induce Langhans-type cells (LGC) in in vitro MGC formation system. Although the exact mechanisms of in vitro MGC formation remains unknown, receptors such as P2X7, integrins, CD98, and macrophage fusion protein are considered to be involved in cell-to-cell adhesion and subsequent fusion process. Monocytes from sarcoidosis patients expressed higher levels of P2X7 and had a higher ability to induce MGC than those from healthy controls. Attributable cells for the formation were CD14+CD16− monocytes. Therefore, CD14+CD16− monocytes may infiltrate into sarcoidal lesions and be fused to form LGC by inflammatory mediators and MDP derived from the pathogens of the disorder. Effective agents for sarcoidosis such as tranilast, allopurinol, and captopril inhibited in vitro MGC formation through inhibiting the expression of adhesion molecule and purinergic receptor. Thus, an in vitro MGC formation model would be a useful tool to understand the relevance of MGC in granulomatous disorders.

© 2002 Japanese Society for Investigative Dermatology Elsevier Science Ireland Ltd. All rights reserved.

1. Introduction

Sarcoidosis is a systemic granulomatous disease characterized by non-caseating granulomas. It is generally recognized that sarcoidosis is a disorder of T lymphocyte-mediated inflammatory response to the unknown antigenic stimuli [1]. Infective and environmental agents have been implicated as such stimuli. In particular, mycobacteria and propionibacteria have been received in much attention as the etiologic agents of sarcoidosis. Ishige et al. [2] found many propionibacteria DNA in all examined 15 Japanese patients with sarcoidosis but not in control subjects using quantitative polymerase...
chain reaction (PCR) on biopsied lymph nodes. These findings were recently confirmed in European patients [3].

For the accumulation of T lymphocytes in sarcoidal lesions, chemoattractant factors released from monocyte-macrophage lineage cells are important, such as regulated on activation normal T expressed and secreted (RANTES) and interleukin-16 (IL-16) [4,5]. Alveolar macrophages from patients with sarcoidosis also express a great variety of cytokines such as tumor necrosis factor-α (TNF-α), IL-6 and IL-12 [6]. Furthermore cutaneous and alveolar macrophages from sarcoidosis exhibit the high density of class II molecules and the adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) [7], suggesting that antigen-presenting function of macrophages are enhanced in patients with sarcoïdosis. Finally, epithelioid cells and multinucleated giant cells (MGC), main component cells of the mature sarcoidal lesions, are derived from monocyte-macrophage lineage cells. Thus, monocyte-macrophage lineage cells are key cells in the initiation, development and maintenance of sarcoidal granulomatous lesions.

There have been a large variety of in vitro granuloma models to understand a role of monocyte-macrophage lineage cells in granulomas, using schistosome egg [8], dextran [9,10], antigen-cytocyte-macrophage lineage cells in granulomas, using granuloma models to understand a role of monocytic lineage of sarcoidal granulomatous lesions. Thus, monocyte-macrophage lineage cells are key cells in the initiation, development and maintenance of sarcoidal granulomatous lesions.

2. Langhans-type and foreign body-type MGC in granulomatous disorders

The presence of MGC in the tuberculous granuloma was first described by Langhans in 1868 [13]. MGC have been thereafter observed in a variety of infectious and uninfected granulomatous disorders. There are several ideas for the functional role of MGC in granulomas, including a disposal function for effete macrophages [14] and specific functions in the phagocytosis of pathogens involved in granuloma formation. Morphologically MGC are classified into Langhans-type cells (LGC) and foreign body-type cells (FGC). LGC show a circular peripheral arrangement of nuclei and FGC have the nuclei scattered in an irregular fashion throughout the cell. LGC are often seen in many infectious granulomatous disorders such as tuberculosis and leprosy, or in unknown pathological inflammatory granulomatous disorders such as sarcoidosis, while FGC are characteristic cells in foreign body granulomas.

Cutaneous involvement of sarcoidosis is classified into specific granulomatous lesions and non-specific reactive lesions such as erythema nodosum. The specific lesions include nodular type, plaque type, lupus pernio, subcutaneous type, and other rare types of lesions such as ichthyosis, psoriatic lesions, vitiligo, and lichenoid eruptions. Scar infiltration is another granulomatous lesions of sarcoidosis in which foreign bodies are present. When histologically and immunohistologically examined, cutaneous sarcoidal lesions had both types of MGC, with predominance of LGC, regardless of the presence or absence of foreign bodies [15]. The number of nuclei in FGC was significantly higher than in LGC. This supports the idea by Van der Rhee et al. [16] that LGC may be precursors of FGC from the standpoint of nuclear number. Surface antigens of monocyte-macrophage lineage cells and adhesion molecules showed the same expression profiles between LGC and FGC: CD1a, CD11a, CD11b, CD11c, CD14, CD16, CD36, CD54, CD68, CD86, MAC387 and 3A5 cytoplasmic. These immuno-histochemical results suggest that both types of MGC may be functionally as well as phenotypically same cells with different distribution of nuclei. However, as shown in the following section, in vitro MGC formation models show that LGC and FGC are formed by different fusion mechanisms [17–20]. Expression of mRNAs for IL-1β and IL-6 has been reported to be different between both cells in Nippostrongylus brasiliensis-induced MGC [21]. Therefore, despite the understanding of their morphological features, it is not determined whether there is any difference in the cellular nature and functional significance of these two types of MGC.

3. In vitro formation of MGC

MGC can be formed in vitro from human blood monocytes by various stimuli [14,17–20,22–40] (Table 1)(Fig. 1). Among several cytokines which are expressed in sarcoidal lesions and considered to be involved in development and maintenance of the lesions [41], IL-1, IL-3, granulocyte-macrophage-colony stimulating factor (GM-CSF) and in-
terferon-γ (IFN-γ) are also associated with MGC formation in vitro. Particularly IFN-γ was reported to be one of the pivotal factors promoting monocyte fusion. When antibodies against IFN-γ were administered, MGC formation was inhibited in vitro [26,28] as well as in vivo [42]. Interestingly, McNally and Anderson [18] described that IFN-γ-induced predominantly LGC together with IL-3 or GM-CSF. Byrd [20] also reported that the cytokine combination of IFN-γ and IL-3 promoted LGC and that dense growth of Mycobacterium tuberculosis surrounded by a ring of nuclei localized to the center of LGC. On the other hand, McNally and Anderson [18] described that IL-4 induced only FGC from monocytes and that addition of IL-3 or GM-CSF optimized the effect of IL-4. McInnes and Rennick [43] also reported that IL-4 promoted FGC formation from murine bone marrow macrophages cultured with IL-3. The importance of IL-4 in FGC formation was confirmed on implanted biomaterial in mice by inhibitory effects of anti-IL-4 antibody on the formation [44]. Interestingly, mRNA and protein concentrations of IL-4 were undetectable in bronchoalveolar lavage fluids from patients with sarcoidosis [45]. DeFife et al. [19] reported that IL-13 has the ability to fuse monocyte-derived macrophages as potently as IL-4 under identical culture conditions, and results in FGC formation. Thus, some cytokines have a trend to induce LGC and others readily induce FGC. The balance of these cytokines produced locally in granulomatous lesions may decide which types of MGC are induced.

Since granulomas occur in the tissues with unsolved infective or uninfective agents, not only inflammatory mediators but a factor derived from such agents may be required for the induction of MGC. Muramyl dipeptide (MDP) is a part of the common structure of bacterial cell walls, a peptidoglycan portion, present in M. tuberculosis and Propionibacterium acnes and detected in MGC in

### Table 1  Reported monocyte-derived multinucleated giant cells

<table>
<thead>
<tr>
<th>Stimulants</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNa of monocytes</td>
<td>Black et al. [22]</td>
</tr>
<tr>
<td>Tetanus toxoid, PPD, NaIO4, PHAb</td>
<td>Postlethwaite et al. [23]</td>
</tr>
<tr>
<td>Con Ac</td>
<td>Kreipe et al. [24]</td>
</tr>
<tr>
<td>PMAa, IFN-γ</td>
<td>Hassan et al. [25]</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Most et al. [26]</td>
</tr>
<tr>
<td>IFN-γ, IL-3</td>
<td>Enelow et al. [17]</td>
</tr>
<tr>
<td>IL-4</td>
<td>Takashima et al. [27]</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Fais et al. [28]</td>
</tr>
<tr>
<td>IL-4</td>
<td>Kazazi et al. [29]</td>
</tr>
<tr>
<td>Con A and/or IFN-γ</td>
<td>Falzoni et al. [30]</td>
</tr>
<tr>
<td>IL-4</td>
<td>McNally et al. [18]</td>
</tr>
<tr>
<td>PHAb</td>
<td>Liu et al. [31]</td>
</tr>
<tr>
<td>IL-4+ M-CSF</td>
<td>Akagawa et al. [32]</td>
</tr>
<tr>
<td>Nippostrongylus brasiliensis SNa of Con A-stimulated mononuclear cells</td>
<td>Seitzer et al. [21]</td>
</tr>
<tr>
<td>Heat-killed candida albicans</td>
<td>Heinemann et al. [33]</td>
</tr>
<tr>
<td>IL-4+ GM-CSF</td>
<td>Dugast et al. [34]</td>
</tr>
<tr>
<td>PMAa</td>
<td>Merrill et al. [35]</td>
</tr>
<tr>
<td>IL-4, IL-13</td>
<td>DeFife et al. [19]</td>
</tr>
<tr>
<td>IFN-γ, IL-3</td>
<td>Byrd [20]</td>
</tr>
<tr>
<td>IL-13 or IL-4+ M-CSF</td>
<td>Ikeda et al. [36]</td>
</tr>
<tr>
<td>IL-4</td>
<td>Jenney et al. [37]</td>
</tr>
<tr>
<td>SNa of HIV-transfected T cells + BCG</td>
<td>Gasser et al. [38]</td>
</tr>
<tr>
<td>SNa of Con A-stimulated mononuclear cells + MDP</td>
<td>Mizuno et al. [39]</td>
</tr>
<tr>
<td>IL-4</td>
<td>McNally et al. [40]</td>
</tr>
</tbody>
</table>

a  SN, supernatants.
b  PHA, phytohemagglutinin-A.
c  Con A, concanavalin A.
d  PMA, phorbol myristate acetate.
lymph nodal [46] and cutaneous lesions of sarcoidosis (Fig. 2). It has been reported that MDP causes macrophage activation [47–50] and produces in animals [51,52] massive epithelioid granulomas indistinguishable from those produced by tubercle bacilli when injected in the form of Freund-type water in oil emulsion [50]. In vitro examination showed that MDP augmented the expression of adhesion molecules on monocytes [53]. When monocytes were cultured with MDP in a MGC formation system induced by supernatants from lectin-stimulated lymphocytes (the conditioned medium) [23,26], the formation of LGC but not FGC was significantly augmented. However, a nonadjuvant analogue of MDP, N-acetylmuramyl-L-alanyl-L-isoglutamine, had no effect on MGC formation. When anti-IFN-γ, anti-IL-3 or anti-GM-CSF mAb was concomitantly added to the culture of monocytes treated with supernatants alone or supernatants with MDP, anti-IFN-γ mAb completely abrogated MGC generation and both anti-GM-CSF and IL-3 mAbs inhibited LGC generation. These findings indicated that IFN-γ is necessary to induce MGC and that IL-3 and GM-CSF are important factors for induction of LGC. However, since about 30% of fusion rate of LGC was still observed when GM-CSF or IL-3 was added in the culture with MDP, other LGC-related cytokines may be released from monocytes stimulated with MDP together with conditioned medium.

4. Subpopulation of monocytes and origin of MGC

Several lines of cells have been utilized for in vitro MGC formation, including human monocytes, macrophages, bone marrow cells [54], monoblastic cell line [55], murine bone marrow cells [43], and rat alveolar macrophages [56]. In human, MGC are considered to be more easily formed from monocytes than macrophages. Most et al. [14] reported that cells differentiated to macrophages by long-term culture or by culture with human serum had less ability to fuse with each other than freshly isolated monocytes. However, macrophages are still able to fuse with freshly isolated monocytes. Antimicrobial activity of monocytes declines during maturation into macrophages. Therefore, they speculated that fusion with monocytes could be of benefit for macrophages that take up certain bacteria or parasites in infectious granulomas. Fais and Pallone [57] reported that virtually no MGC were formed from intestinal macrophages in con-

---

**Fig. 2** Muramyl dipeptide in cutaneous lesions of sarcoidosis. Acetone-fixed cryostat sections of sarcoidal cutaneous lesion 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 anti-muramyl dipeptide antibody (kindly gifted from Dr. Eishi) for 60 min and then biotinylated goat anti-rabbit 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. Muramyl dipeptide was present in MGC (arrows).
contrast with autologous peripheral monocytes. Their group also showed that MGC are highly induced from HIV-1-infected monocytes than macrophages [58] and suggested that MGC formed in the brain and lymph nodes of AIDS patients may result from the fusion of HIV-1-infected blood monocytes recruited and activated in the tissues. We [39] confirmed their findings using CSF-induced macrophages; i.e. when using M-CSF- or GM-CSF-induced macrophages as precursor cells of MGC, the formation was extremely suppressed compared to monocytes. In sarcoidal lesions both FGC and LGC reacted with anti-monocyte-macrophage markers such as CD14, CD11a and 3A5, but not with antimacrophage markers, such as CD16 and MAC387. MGL, a macrophage endogenous calcium-type lectin and a specific marker for tissue macrophages [59], was also negatively stained in both MGC and epithelioid cells [15]. These results, in consistent with findings of in vitro MGC formation system, suggest that MGC in sarcoidal granulomas are derived from monocytes but not from tissue macrophages.

Monocytes fall into subpopulations comprising CD14+/CD16– and CD14+CD16+ cells [60]. CD14+/CD16– cells are the major population and CD14+CD16+ cells are regarded as proinflammatory monocytes. The mRNA levels for IL-10, phagocytosis, and reactive-oxygen production in CD14+CD16+ cells are lower than those in CD14+/CD16– cells [61]. On the other hand, CD14+CD16+ monocytes efficiently produce TNF-α [50]. We [39] previously reported that MGC formation rate was the same between freshly isolated whole monocytes and CD14+/CD16– monocytes (Fig. 3). On the other hand, MGC were not produced from CD14+/CD16– monocytes. Therefore, CD14+CD16+ cells, a major population of monocytes, are responsible for MGC formation.

5. Mechanism of in vitro formation of MGC

The exact mechanism of in vitro and in vivo MGC formation is not determined. Cell fusion may be mediated by plasma membrane receptors that are responsible for cell-to-cell adhesion and their fusion process [62].

P2X7 is considered to be one of such receptors. P2X receptors are ligand-gated ion channels that are activated by extracellular ATP. P2X7 belongs to the P2X receptor family and is a bifunctional receptor [63]. Whereas transient stimulation with extracellular ATP allows this receptor to behave as a typical cation-selective ion channel permeable to K+, Na+, and Ca2+, repetitive stimulation allows it to change to a non-selective pore that allows transmembrane fluxes of hydrophilic molecule [30] and prolonged activation of P2X7 leads unavoidably to cell death. In monocyte-macrophage

---

**Fig. 3** Multinucleated giant cells formation by whole, CD14+/CD16–, or CD14+CD16+ monocytes. Whole monocytes (A) or isolated CD14+/CD16–, or CD14+CD16+ monocytes (B) were cultured for 4 days in RPMI1640 supplemented with a final concentration of 50% supernatants of concanavalin A-stimulated mononuclear cells. Fusion index (%) is determined as (the total number of nuclei within MGC)/(the total number of nuclei counted) × 100.
lineage cells, P2X7 has been associated with cytotoxicity, maturation, and release of IL-1β [64,65], but it has been suggested that it also might participate in MGC formation [66]. Falzoni et al. [30,62] reported that formation of concanavalin A-induced MGC was inhibited by oxidized ATP, a P2X7 blocker, or an anti-P2X7 monoclonal antibody. On the other hand, cell fusion is augmented by addition of apyrase or hexokinase which destroy extracellular ATP [62]. Macrophage cell clones expressing P2X7 to a very high level spontaneously fuse during in vitro culture, whereas clones selected for lack of P2X7 never do [66]. Since P2X7 is preferentially localized at site of cell-to-cell contact and does not act as a chemotactic or cell adhesion receptor, it is speculated that P2X7 is involved in the generation of a fusion pore which is the very last step of MGC formation [62,67].

Adhesion molecules such as LFA-1 and ICAM-1 are also considered to be involved in cell fusion, because MGC formation was blocked by anti-LFA-1 and anti-ICAM-1 antibody [29]. Indeed, the change in ICAM-1 expression and cellular distribution has been observed in the IFN-γ-induced MGC [28]. The importance of β1 integrin family has been stressed as another fusion-associated adhesion molecule. McNally and Anderson [40] documented that initial monocyte adhesion is mediated by β2 integrins, while during the induction of macrophage fusion by IL-4, an additional dependence on β1 integrins is acquired. β2 Integrins are present on monocyte after initial adhesion and are strongly expressed on fusing macrophages. In contrast, β1 integrins are not detected on monocyte but begin to appear during macrophage development and are strongly expressed on fusing macrophages and MGC.

CD98, initially termed fusion regulatory protein-1, has been reported to participate in MGC formation of monocytes as well as in virus-mediated cell fusion [68]. Anti-CD98 antibodies induced tartrate-resistant acid phosphatase+ MGC formation from monocytes in which process ADAM 9 was recently reported to be involved [69].

More recently, macrophage fusion protein (MFR) was cloned from rat alveolar macrophages by Vignery’s group [70]. CD47 is a ligand for the receptor and MFR and CD47 interact via their immunoglobulin V domain during macrophage fusion [71]. They also reported that CD44, an integral membrane glycoprotein, is involved in macrophage fusion [56]. MFR and CD44 have been evidenced to be induced at the onset of macrophage fusion [70].

6. Ability of MGC formation in sarcoidosis patients

Using in vitro MGC formation system, we [72] examined the ability of monocytes from patients with sarcoidosis to produce MGC. Monocytes from sarcoidosis patients had a higher ability to form MGC than those of control subjects (Fig. 4). The high fusion rate in sarcoidosis patients was due mainly to the enhancement of LGC formation. These findings suggest that there is the potential activity of peripheral monocytes to easily produce MGC, in particular LGC by inflammatory stimuli in sarcoidosis. The heightened ability to form MGC was also reported in monocytes of patients with Crohn’s disease [31]. On the other hand, there was no difference in the ability of the conditioned medium to produce MGC between sarcoidosis patients and healthy subjects. Indeed, there was no difference in protein levels of MGC-induced cytokines such as IFN-γ, IL-3, and GM-CSF in the conditioned medium between these two groups.
BzATP, a P2X7 receptor antagonist, induced more cell lysis of monocytes cultured in conditioned medium for 24 h from sarcoidosis patients than cells from healthy controls [72]. This indicated that monocyte-macrophage lineage cells in sarcoidosis patients expressed higher levels of P2X7 receptor than those in healthy controls.

7. Influence of MGC formation by effective drugs on sarcoidosis

The first therapeutic approach for sarcoidal skin lesions is to use topical corticosteroid. When such a topical medication produces low responses, various systemic treatments are adopted, including corticosteroid and cytotoxic drugs. However, since the use of such immunosuppressive agents is limited because of their side effects, safer medications are tried and some of them have been reported to be effective, including tranilast, allopurinol, Angiotensin I-converting enzyme (ACE) inhibitors, and anti-malarial agents. Tranilast is a widely used antiallergy drug in Japan and adopted for the treatment of atopic dermatitis patients [73]. Allopurinol is an inhibitor of xanthine oxidase [74] and captopril is an inhibitor of ACE. All three agents have been reported to have beneficial effects on some patients with sarcoidosis and other granulomatous diseases [75–78], although the action mechanisms are not known. When added into culture of monocytes with supernatants from Con A-stimulated lymphocytes, these three agents inhibited the formation of MGC and down-regulated the expression of ICAM-1 of monocytes [79,80]. On the other hand, the susceptibility of monocytes to BzATP-mediated cytolysis was significantly higher in monocytes treated with allopurinol and captopril than untreated monocytes, while tranilast did not exhibit any effect on LDH release. Thus, although allopurinol or captopril, and tranilast have different effects on cell surface molecules involved in MGC formation, they are considered to exert beneficial effects on granulomatous disorders by direct actions on monocyte-macrophage lineage cells partly through the down-regulation of ICAM-1 and P2X7 receptors.

8. Prospectives

We have reviewed in vitro MGC formation induced from human monocytes. Although such an in vitro MGC formation model is a useful tool to understand the molecular mechanisms of cell-to-cell fusion, many questions with respect to granulomas remain to be addressed. Although MGC are recognized to play a crucial role in granulomatous inflammation, the precise functions of LGC and FGC are still unknown. Secondarily, the ability of monocytes to induce MGC was enhanced in sarcoidosis patients through P2X7 receptors, a key factor in the late step of cell fusion. However, it is not determined whether other fusion-associated molecules such as CD98, MFR, and CD47 are involved in granulomatous disorders. Finally, the most important issue to be solved is what epithelioid cells are. Epithelioid cells are the main component cells in the granulomatous lesions, but their properties and differentiation process are not clearly understood.

Acknowledgements

This work was supported by grants-in aid from the Ministry of Education, Science, and Culture of Japan (11670855, 13670903) and by a grant from Kansai Medical University (research grant D).

References


Monocyte-derived multinucleated giant cells and sarcoidosis


