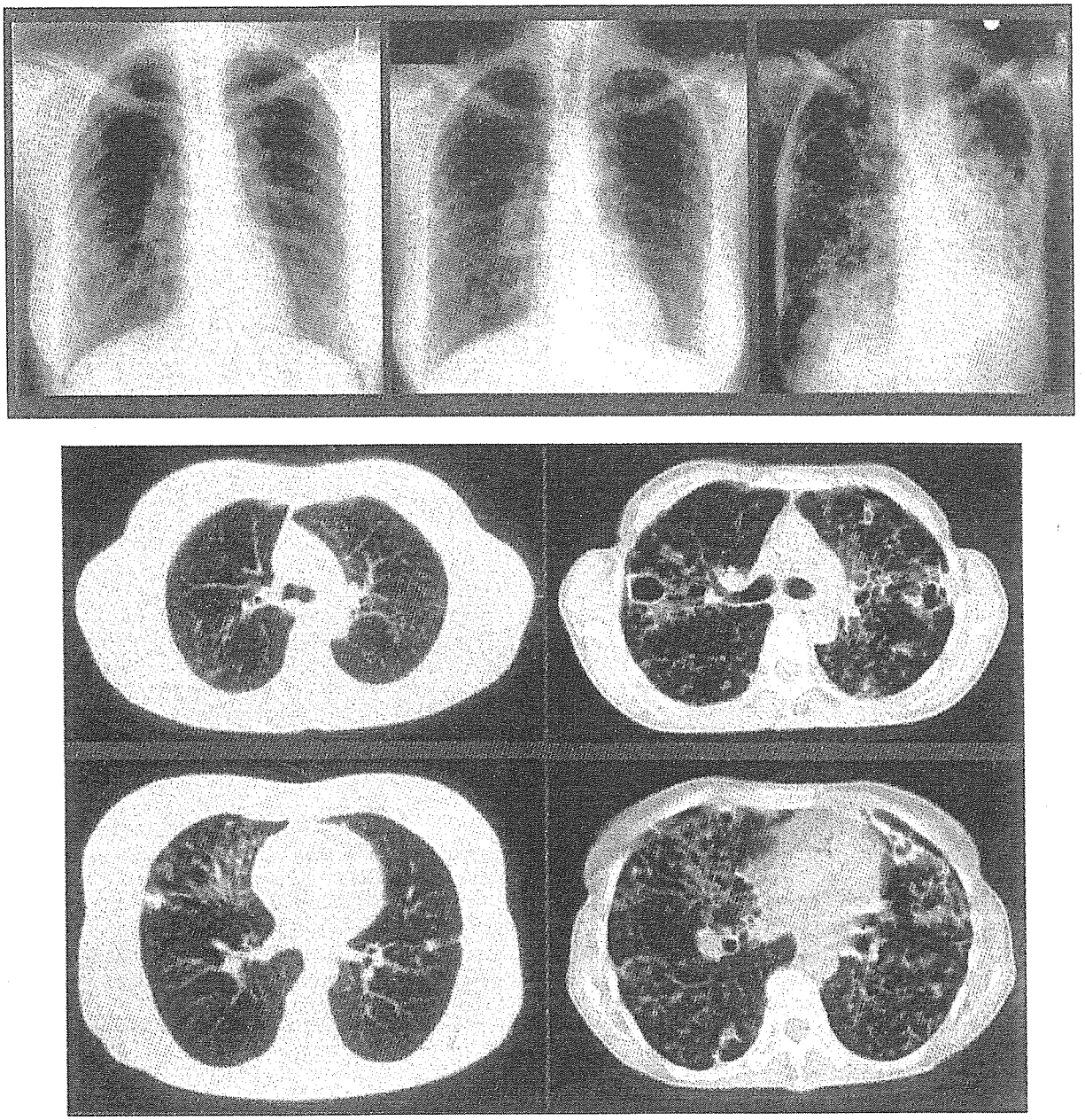

FIG. 4. Chronological changes on chest radiograms preceding the MAC serovar 4-related death of a patient whose initial radiographic finding was a bronchiectasis lesion. (Top left) Unilateral bronchiectasis lesion at the age of 37; (top middle) left unilateral small cavitary lesion and bilateral bronchiectasis lesions at the age of 48; (top right) bilateral cavitary lesions with aggravation of the bilateral bronchiectasis lesions at the age of 57; (bottom) Chest computed tomograms at the ages of 51 (right panels) and 56 years (left panels).

of >8 μg/ml), 13% for CAM (MICs of >32 μg/ml), and 17% for sparofloxacin (MICs of >4 μg/ml) were categorized as resistant in the serovar 4 group (12). On the other hand, in the *M. intracellulare* serovar 14 and 16 group, most isolates were categorized as susceptible or moderately susceptible to EB, RFP, CAM, or sparofloxacin. The range of MICs of each drug for isolates in the *M. avium* serovar 1, 6, 8, and 9 group was also found to be broad.

DISCUSSION

MAC diseases have attracted attention because they can develop as opportunistic infections and cause death in AIDS patients. However, the prognosis of pulmonary MAC disease in HIV-negative cases is still unclear, and therapeutic approaches had not yet been established in 1997 (2). Furthermore, when we started this prospective study in 1990, it was

TABLE 3. Causes of non-MAC-related deaths (n = 13)

Case	Serotype	Age (yr) at death	Cause of death[a]	Outcome of MAC treatment	Change in radiographic findings	Extent of lesions
1	4	73	Lung cancer	Cured	None	Minimal
2	4	84	Liver cirrhosis	Relapsed	Worsened	Moderate
3	4	67	Liver cirrhosis	Cured	None	Minimal
4	6	81	IHD	Cured	Improved	Minimal
5	8	72	IPF	Nonresponsive	Worsened	Moderate
6	9	76	IPF	Nonresponsive	None	Minimal
7	14	76	IHD	Nonresponsive	Worsened	Far
8	16	73	Stomach cancer	Nonresponsive	Improved	Far
9	16	87	Cerebral infarct	Relapsed	Worsened	Minimal
10	16	91	Cerebral infarct	Relapsed	Worsened	Moderate
11	16	83	Parkinson's disease	Cured	Improved	Minimal
12	1	84	Sudden death at home	Relapsed	Worsened	Far
13	1	65	RA, pneumonia	Nonresponsive	None	Moderate

[a] IHD, ischemic heart disease; IPF, idiopathic pulmonary fibrosis; RA, rheumatoid arthritis.

probably not known that there were some pulmonary MAC patients with poor prognosis among immunocompetent patients. The evaluation of prognostic predictors and treatment outcomes for pulmonary MAC disease requires long-term follow-up of many patients. In this study, we have been able to follow 68 patients on a monthly basis for more than 5 years. Most of the patients had undergone multidrug antituberculosis chemotherapy, which was conventionally used before the American Thoracic Society recommendation of 1997 (1, 2). Half of the patients had also been treated with CAM in addition to being treated with multidrug antituberculosis chemotherapy. Consequently, the rate of sputum-negative conversion was similar to that in the previous reports (15, 22, 26, 30). At least 21 (30.9%) of 68 patients with pulmonary MAC disease experienced progression or aggravation of the disease and died, excluding non-MAC-related deaths, because the subjects were older. In considering the prognosis of MAC disease in the HIV-negative patient, the progression or aggravation of pulmonary MAC disease that was not due to an opportunistic infection was a direct cause of death.

The ratio of deaths unrelated to MAC was high in the group with serovars other than serovar 4. This was especially true for the serovar 16 patients, who were significantly older than the patients with serovar 4. The 13 cases of deaths unrelated to MAC, including six patients with worsening findings on radiograms, were excluded from the survival prognosis analysis in order to achieve accurate evaluation. Although the number of cured patients was also small, the population in our study seemed to be appropriate to evaluate the prognostic predictors in patients with pulmonary MAC disease.

Regarding the correlation between treatment outcomes and survival prognosis, it is first very important that all cured patients, including one with serovar 8 who underwent surgical therapy, were alive at the end of this study. Second, it is important that the relapsed patients had a significantly better prognosis than the nonresponders to treatment. The extent of radiographic lesions was also significantly correlated with the prognosis of survival. These results reveal that the outcomes of chemotherapy are closely associated with the survival time for patients with pulmonary MAC disease. The severity of disease and the outcome of chemotherapy could also be prognostic factors for survival in pulmonary MAC patients.

We also studied the relationship between the serotypes of MAC isolates and the long-term survival of patients with pul-

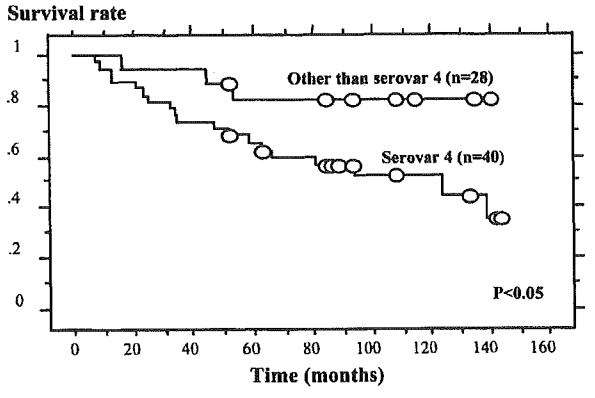

FIG. 5. Kaplan-Meier survival analysis of MAC patients with serovar 4 and those with other serovars. Log rank (Mantel-Cox) test, $P < 0.05$.

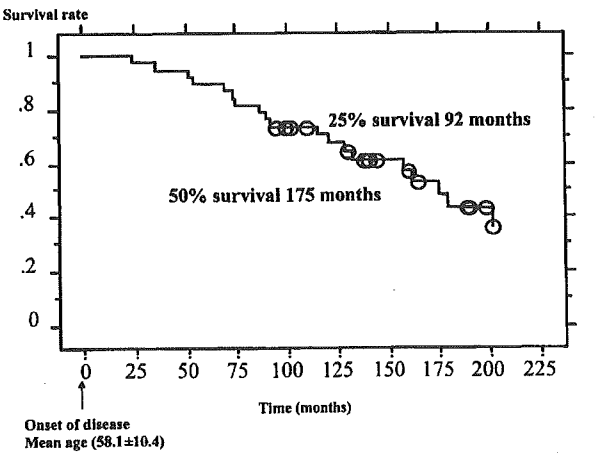

FIG. 6. Kaplan-Meier survival analysis of MAC patients with serovar 4 from the onset of disease.

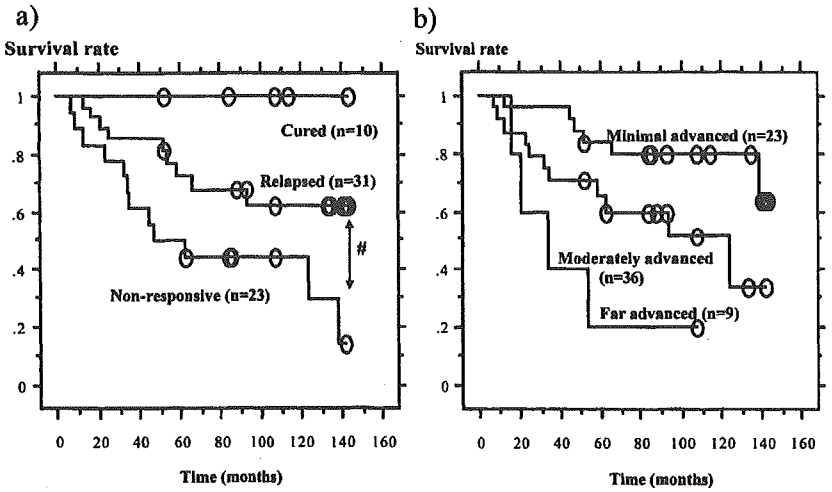

FIG. 7. Kaplan-Meier survival analysis of patients with pulmonary MAC disease divided by (a) bacteriological outcome and (b) the extent of radiographic lesions. (a) Log rank test, $P < 0.05$ (#); (b) log rank (Mantel-Cox) test, $P < 0.01$.

monary MAC disease. It was more difficult to convert sputum to negative when the disease was caused by serovar 4 than for other serovars. The serovar 4 isolates were more resistant to RFP and CAM, and the patients with serovar 4 were more frequently nonresponders to the multidrug chemotherapy than patients with other serovars. However, the sputa of most patients with pulmonary MAC disease became positive on culture during the follow-up period, and the rates were nearly equal between the serovar 4 and the other-serovar groups. This might be due to the factors involved in long-term follow-up and the higher relapse rate in the population of patients being retreated with chemotherapy than in those receiving initial treatment. The patients also may acquire subsequent infections from the living environment, and we have begun a study to investigate MAC organisms from the houses where the patients live.

The radiographic findings for the serovar 4 group had also become significantly worse in the follow-up periods compared with those in the other-serovar group. In cases of death, the radiographic findings for patients with upper-lobe cavitary disease or with nodular bronchiectasis at enrollment had progressed to bilateral cavitary lesions with sclerofibrotic and emphysematous changes (Fig. 2 to 4). Although radiographic findings alone, whether cavitary or bronchiectasis, cannot be a prognostic tool, the progress of the disease should be closely observed by means of radiographic changes.

In conclusion, the survival time of the serovar 4 group was significantly shorter than that of the other-serovar group. At an

TABLE 4. Susceptibilities of patient isolates to ethambutol, rifampin, clarithromycin, and sparofloxacin

Drug, species, and serovar	No. of isolates with MIC (μg/ml) of:							
	≤0.78	1.56	3.13	6.25	12.5	25	50	≥100
Ethambutol								
M. avium serovar 4		1	1	8	11	7	2	
M. avium serovars 1, 6, 8, 9			1	3	3	2		
M. intracellulare serovars 14, 16		1	2	4	1			
Rifampin								
M. avium serovar 4	3		4	6	9	4	4	
M. avium serovars 1, 6, 8, 9	1	2	1	2	2	1		
M. intracellulare serovars 14, 16		1	5	2				
Clarithromycin								
M. avium serovar 4	6	5	4	10	1			4
M. avium serovars 1, 6, 8, 9	2	1	3	1	1			
M. intracellulare serovars 14, 16		3	5					
Sparofloxacin								
M. avium serovar 4	15	7	3	3	1	1		
M. avium serovars 1, 6, 8, 9	4	1	2	1				
M. intracellulare serovars 14, 16		5	3					

early stage of infection, it is difficult to judge the prognosis of patients with pulmonary MAC disease. Although further studies with large numbers of patients are required, the identification of serotype-specific GPLs may be helpful for prognosis. However, there were MAC-related deaths in patients with pulmonary MAC disease caused by serovar 8 or serovar 14. The importance of other serovars as prognostic predictors will be analyzed when the number of cases with each serovar increases to a level sufficient for statistical evaluation. There were also a number of nonserotypeable patients, including some of the cases with poor prognoses. Thus, it could be argued that the sensitivity of serotyping should be improved. Also, the laborious and time-consuming serotype identification of isolates used in this study needs to be replaced with simple and rapid diagnostic methods, and serotyping is not routinely performed. We have developed enzyme-linked immunosorbent assay serodiagnostic tests that use various serotypes of glycopeptidolipids as antigens and a new serotyping method which uses high-performance liquid chromatography/mass spectrometry analyses with a small-scale preparation of GPLs from MAC isolates for 2 days (21, 27).

The number of serovars identified was lower than in previous reports (4, 9, 13, 35). This might be due to either the effects of the preceding multidrug chemotherapy or to problems of identification, because only 14 serovar-specific polar GPLs from MAC have been structurally characterized at this time (5, 8, 24). Also, neither the GPL antigen-based enzyme-linked immunosorbent assay nor the original type-specific rabbit antisera were available during our study. However, the serotype specificity could be accurately identified using TLC and FAB-MS analyses. *M. avium* and serotype 4 predominated in this study because of the high geographical distribution of *M. avium* in the Kinki area, in which our institute is located (32). The skewed distribution may also relate to the previous multidrug chemotherapy that most of the enrolled patients had undergone, because the disease caused by *M. avium* was more difficult to cure than *M. intracellulare*-related disease (37). Furthermore, in cases where the serotypes were analyzed twice, both isolates were identified as serovar 4.

The mechanisms underlying resistance to MAC organisms are likely to be highly informative in regard to both host defense against these infections and the basis of the more virulent MAC relatives. It was recognized early in the HIV pandemic that the decline of $CD4^+$ T lymphocyte levels to below 100 cells/mm^3 was a profound risk for the development of disseminated MAC (7, 17). Since the $CD4^+$ T lymphocyte is a major producer of gamma interferon as well as other cytokines, it was speculated that gamma interferon might be important in the control of MAC disease. Despite the wealth of information regarding disseminated infections with MAC, the underlying immune defects have yet to be convincingly demonstrated for pulmonary MAC infections, except in the case of HIV infection. Specific serotypes such as 1, 4, and 8 can be frequently isolated from humans infected with HIV, and the prognosis after infection differs depending on the serotype. Serovar 4 shows an unfavorable prognosis, whereas serovar 16 is associated with rapid recovery (8, 13).

No information dealing on the virulence factor of MAC that is directly related to the intracellular bactericidal activity has been available to date. We have previously reported effects of (i) various GPLs purified from MAC on the phagocytic processes of human polymorphonuclear leukocytes, (ii) GPL-coated heat-killed staphylococcal cells that were phagocytosed by polymorphonuclear leukocytes, and (iii) the phagosome-lysosome (P-L) fusion (34). Phagocytosis was strongly promoted and the P-L fusion was markedly inhibited by serovar 4, but not serovar 16, GPLs. Serotype 8 GPLs showed concomitant stimulation of both phagocytosis and P-L fusion. These effects may be due to an unknown interaction between specific carbohydrate chains and host phagocytic cell membranes.

The pulmonary MAC disease caused by serovar 4 had a poor long-term survival prognosis. For the treatment of pulmonary MAC disease, including surgery, diagnosis and treatment at an early stage are important. In cases of patients with pulmonary MAC disease caused by serovar 4, it was argued that multichemotherapy including CAM (one of the newer macrolides) and sparofloxacin (one of the newer fluoroquinolones) should be prescribed, because the results of MIC determinations showed that CAM and sparofloxacin were more effective against serovar 4 than EB or RFP. Furthermore, lung resections should be performed in patients with adequate pulmonary reserve for whom medical therapy has been unsuccessful. In addition to the defense mechanism in the patient's lung, the virulence of the organism, and resistance to chemotherapy, a problem regarding the worsening of pulmonary MAC disease that also needs to be addressed is patient compliance issues, which may include a lack of knowledge of the gravity of the disease on the part of both the patient and the physician.

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Macrophages in Inflammation

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Abstract: The inflammatory process is usually tightly regulated, involving both signals that initiate and maintain inflammation and signals that shut the process down. An imbalance between the two signals leaves inflammation unchecked, resulting in cellular and tissue damage. Macrophages are a major component of the mononuclear phagocyte system that consists of closely related cells of bone marrow origin, including blood monocytes, and tissue macrophages. From the blood, monocytes migrate into various tissues and transform macrophages. In inflammation, macrophages have three major function; antigen presentation, phagocytosis, and immunomodulation through production of various cytokines and growth factors. Macrophages play a critical role in the initiation, maintenance, and resolution of inflammation. They are activated and deactivated in the inflammatory process. Activation signals include cytokines (interferon γ, granulocyte-monocyte colony stimulating factor, and tumor necrosis factor α), bacterial lipopolysaccharide, extracellular matrix proteins, and other chemical mediators. Inhibition of inflammation by removal or deactivation of mediators and inflammatory effector cells permits the host to repair damages tissues. Activated macrophages are deactivated by anti-inflammatory cytokines (interleukin 10 and transforming growth factor ß) and cytokine antagonists that are mainly produced by macrophages. Macrophages participate in the autoregulatory loop in the inflammatory process. Because macrophages produce a wide range of biologically active molecules participated in both beneficial and detrimental outcomes in inflammation, therapeutic interventions targeted macrophages and their products may open new avenues for controlling inflammatory diseases.

INTRODUCTION

Inflammation is a complex, highly regulated sequence of events that can be provoked by a variety of stimuli including pathogens, noxious mechanical and chemical agents, and autoimmune responses. The subsequent cascade of events is characterized by the signs and symptoms of redness, swelling, heat, and pain. The inflammatory response occurs in the vascularized connective tissue, including plasma, circulating cells, blood vessels, and cellular and extracellular components. This corresponds with increased microvascular caliber, enhanced vascular permeability, leukocyte recruitment, and release of inflammatory mediators [1]. Inflammation is the primary process through which the body repairs tissue damage and defends itself against stimuli. In the physiologic condition, the regulated response protects against further injury and clears damaged tissue. In pathologic situation, inflammation can result in tissue destruction and lead to organ dysfunction.

The process of inflammation is divided into acute and chronic patterns. Acute inflammation is of relatively short duration, lasting for minutes, several hours, or a few days, and its main features are the exudation of fluid and plasma proteins (edema) and the emigration of leukocytes, predominantly neutrophils. Chronic inflammation is of longer duration and is associated histologically with the presence of lymphocytes and macrophages, the proliferation of blood vessels, fibrosis, and tissue necrosis. Many factors participate in the course and histologic features of both acute and chronic inflammation. In inflammation, macrophages have three major function; antigen presentation, phagocytosis, and immunomodulation through production of various cytokines and growth factors [2, 3]. No human has been identified as having congenital absence of this cell line, probably because macrophages are required to remove primitive tissues during fetal development as new tissues develop to replace them. In this article, we will review the role of macrophages in inflammation.

DEVELOPMENT OF MACROPHAGES

The mononuclear phagocyte system consists of cells that have a common lineage whose primary function is phagocytosis. Monocytes and tissue macrophages in their various forms make up the system [4]. These cells are a system because of their common origin, similar morphology, and common functions, particularly phagocytosis. The cells of the mononuclear phagocyte system originate in the bone marrow, circulate in the blood, and mature and become activated in various tissues (Fig. 1). The first cell type that enters the peripheral blood after leaving the marrow is incompletely differentiated and is called monocytes. When the monocyte reaches the extravascular tissue, it undergoes transformation into a larger phagocytic cell, the macrophage. In addition to augmenting phagocytotic activity, macrophages have the potential of being activated, a process that results in increased cell size, increased production of lysosomal enzymes, more active metabolism, and greater ability to phagocytose and kill ingested microbes. Once they settle in the tissues, these cells mature and become macrophages. Macrophages may exhibit different morphology and functional properties after activation by external stimuli, including microorganisms. Some develop abundant cytoplasm and are called epithelioid cells because the morphologic similarity to epithelial cells of the skin. Activated macrophages can fuse to form multinucleated giant cells. Epitheliod cells and multinucleated giant cells are the major cellular component of granulomas, a typical phenotype of chronic inflammation [5]. Macrophages are found in all organs and connective tissues and named to designate their location, such as microglial cells in the central nervous system, Kupffer cells in the liver, alveolar macrophages in the lung, and osteoclasts in the bone.

FUNCTIONS OF MACROPHAGES

Macrophages have at least three major functions: antigen presentation, phagocytosis, and immunomodulation [2, 3]. The functional responses of macrophages in host defense consist of sequential steps; active recruitment of the cells to the site of infection, recognition of microbes, phagocytosis, and destruction of ingested microbes. In addition, macrophages produce biological active molecules that serve many important roles in innate and adaptive immune responses.

Antigen Presentation

Antigen-presenting cells function to display antigens for recognition by lymphocytes and to promote the activation of lymphocytes. Antigen-presenting cells include dendritic cells and monocytes/macrophages. Macrophages containing ingested microbes present microbial antigens to differentiated effector T lymphocytes. The effector T cells then activate macrophages to kill microbes in association with cytokines. The macrophage-cytokine-T lymphocyte axis plays a critical role in development of cell-mediated immunity against intracellular pathogens, including *Mycobacterium tuberculosis*, *Salmonella typhi*, and *Listeria monocytogenes*. Ingested macrophages play a role in activate/differentiate naïve T lymphocytes to induce primary responses to microbial antigens, although it is likely that dendritic cells act as more effective inducers of the response.

Phagocytosis

Macrophages ingest materials to eliminate waste and debris (scavenging) and to kill invading pathogens. The microbicidal mechanisms of phagocytes are largely confined to intracellular vesicles (lysosomes and phagolysosomes) to protect the cells themselves from injury. Therefore,

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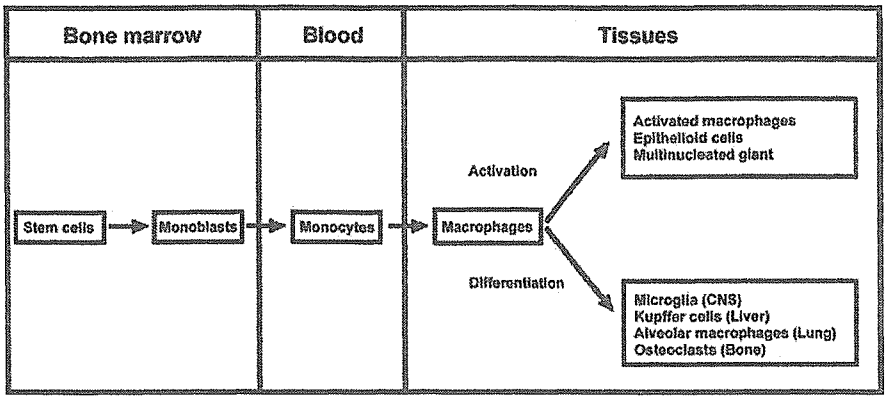

Fig. (1). Maturation of mononuclear phagocytes.

ingestion of microbes into these vesicles is a necessary prelude to microbial killing, and the first step in ingestion is recognition of the microbes by cell surface receptors [6]. Macrophages also express receptors that activate the cells to produce cytokines and microbicidal substances and receptors that stimulate the migration/chemotaxis of the cells to sites of infection. These include mannose receptors, scavenger receptors, receptors for opsonins, seven α-helical transmembrane/G protein-coupled receptors, and Toll-like receptors (TLRs).

The mannose receptors and scavenger receptors function to bind and ingest microbes. The mannose receptor is a macrophage lectin that binds terminal mannose and fucose residues of glycoproteins and glycolipids on microbial cell walls. Because glycoproteins and glycolipids contain terminal sialic acid or N-acetylegalactosamine, the macrophage mannose receptor recognizes microbes but not host cells. Macrophage scavenger receptors bind a variety of microbes as well as modified low-density lipoprotein particles that cannot interact the conventional LDL receptors.

Receptors for opsonins promote phagocytosis of microbes coated with various proteins, including antibodies, complement proteins, and lectins. Fc receptors and complement receptors of macrophages contribute to the opsonin-mediated process. Seven α-helical transmembrane/G protein-coupled receptors are expressed on leukocytes, recognize microbes and certain mediators that are produced in response to infections, and function mainly migration/chemotaxis of leukocytes to sites of infection [7]. These receptors are found on polymorphonuclear neutrophils (PMNs), macrophages, and most other types of leukocytes. The receptors recognize short peptides containing *N*-formylmethionyl residues. Because all bacterial proteins and few mammalian proteins (only those synthesized within mitochondria) are initiated by *N*-formylmethionine, this receptor allows leukocytes to detect and respond to bacterial proteins. PMNs and macrophages also express seven α-helical transmembrane receptors for chemokines, proteolytic products of complement proteins, lipid mediators such as platelet-activating factor, prostaglandin E, and leukotriene B4. Biding of ligands to the receptors induces migration/chemotaxis of cells from the blood through endothelium and production microbicidal substances, including reactive oxygen/nitrogen intermediates (ROIs/RNIs).

TLRs are a family of membrane proteins that serve as pattern recognition receptors for a variety of microbe-derived molecules and stimulate inflammatory/innate immune responses to the microbes expressing pathogen associated molecular patterns (PAMPs) [8]. To date, 10 mammalian TLRs have been identified and are involved in responses to widely divergent types of molecules that are commonly expressed by microbes but not mammalian cells. The innate immune response to one species of microbe may reflect an integration of the responses of several TLRs to different molecules produced by the microbe. The microbial products that stimulate TLRs include lipopolysaccharide (LPS) of gram-negative bacteria (TLR4), peptidoglycan, lipoteichoic acid, and lipoarabinomannan of gram-positive bacteria (TLR2), flagellin (TLR5), and unmethylated CpG motifs (TLR9). Specificities of the TLRs are influenced by non-TLR adapter molecules. For example, LPS first binds to soluble LPS-binding protein (LBP) in the blood or extracellular fluid, and this complex serves to facilitate LPS binding to CD14, which exists as both a plasma protein and a glycophosphatidylinositol-linked membrane protein on most cells except endothelial cells. Once LPS binds to CD14, LBP dissociates, and the LPS-CD14 complex physically associated with TLR4. The predominant signaling pathway used by TLRs results in the activation of nuclear factor (NF)-κB. The genes that are expressed in response to TLR signaling encode proteins important in components of innate immune responses. These include proinflammatory cytokines [interleukin (IL)-1, IL-12, and tumor necrosis factor α (TNF-α)], endothelial adhesion molecules, and proteins involved in microbicidal mechanisms (inducible nitric oxide synthase).

Immunomodulation

Activated macrophages release cytokines such as IL-1, IL-6, TNF-α, interferon (IFN)-α/ß, IL-10, IL-12, and IL-18 that participate in the

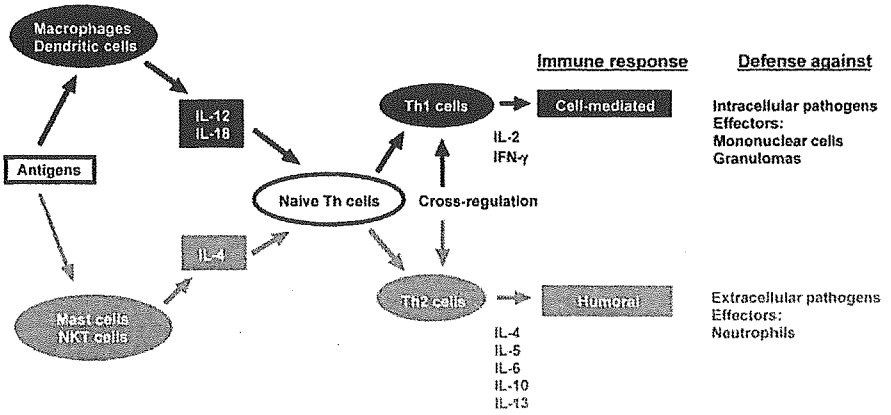

Fig. (2). The system of Th1 and Th2 lymphocytes.

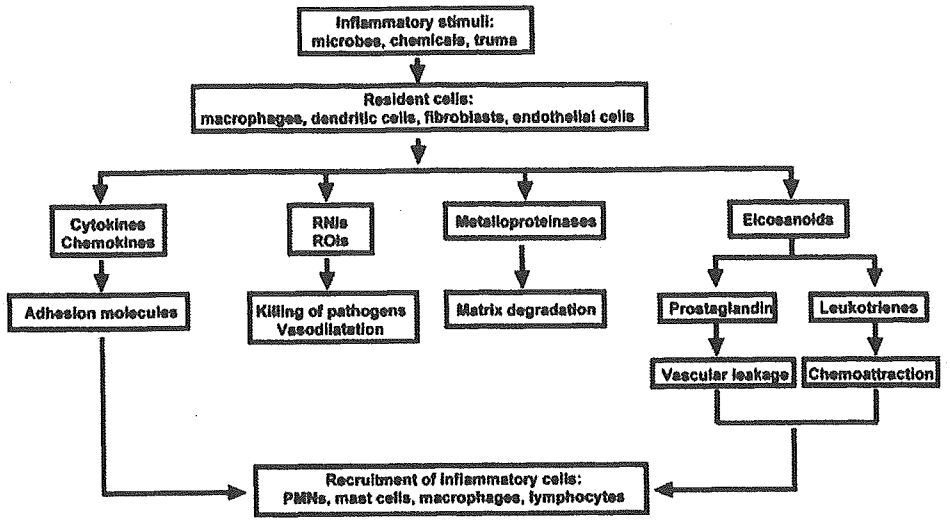

Fig. (3). Initiation of inflammation and recruitment of inflammatory cells.

regulation of immune/inflammatory responses. IL-12 is a heterodimeric cytokine produced primarily by antigen-presenting cells that has important activities in the regulation of various aspects of the immune response. IL-12 stimulates proliferation of activated T and natural killer (NK) cells, enhances NK and lytic activity of cytotoxic T lymphocytes, and induces IFN-γ production by T and NK cells. IL-12 plays a central role in promoting type 1 helper T (Th1) immune responses and thus cell-mediated immunity (Fig. **2**) [9]. IL-12 represents a functional bridge between the early nonspecific innate resistance and the subsequent antigen-specific adaptive immunity [10]. In addition, they produce chemokines that stimulate leukocyte movement and regulation of migration leukocytes from the blood to tissues [7].

INITIATION OF INFLAMMATION

The inflammatory response is a tightly ordered sequence of events. After the initial stimulus, a massive influx of inflammatory cells to the site of injury begins. The process begins with release of chemokines and soluble mediators from locally residing cells, including vascular endothelial cells, dendritic cells, macrophages, and interstitial fibroblasts. Signals from these events alter the local adhesion molecule profile and create a chemotactic gradient that recruits cells from the circulation. PMNs are the first inflammatory cells to extravasate and arrive at the site of injury. Specialized mononuclear cells, such as monocytes/macrophages and lymphocytes, are then recruited by further downstream signals. Macrophages participate in the production, mobilization, activation, and regulation of inflammatory/immune effector cells.

When inflammation is triggered by a pathogen, resident macrophages are stimulated by pattern recognition receptors expressed on macrophages as the innate immune response. These receptors include the TLR family and can recognize molecular structures on microbial pathogens, but not on mammalian cells. TLR-mediated responses lead to the activation of NF-κB in association with a battery of proinflammatory cytokine genes, including IL-1 and TNF-α. Because activated macrophages are the main producers of such inflammatory cytokines, the cells play a key role in the initiation of inflammation (Fig. **3**). In addition to the innate immune response mediated by TLRs, microbes are opsonized by specific antibodies and complement pathways.

ACTIVATION OF MACROPHAGES

The important step in the functional maturation and inflammation of macrophages is the conversion from a resting to an activated macrophage. The term activated macrophage indicates that the cell has an augmented capacity to kill microbes or tumor cells. Activation signals include T lymphocyte-derived cytokines [IFN-γ, granulocyte-monocyte colony stimulating factor (GM-CSF), and TNF-α], microbial products (eg. LPS), immune complexes, chemical mediators, and extracellular matrix proteins such as fibronectin. Activated macrophages exhibit an enhanced capacity to kill microbes and tumor cells. They are larger, with more pseudopods and pronounced ruffling of the plasma membrane, and they produce a wide variety of biologically active products that, if unchecked, result in the tissue damage and fibrosis in chronic inflammation (Table 1). In short-lived inflammation, if the eliciting agent is eliminated, macrophages eventually disappear. In chronic inflammation, macrophage accumulation persists.

Table 1. Upregulated Functions of Activated Macrophages

Microbicidal activity
Tumoricidal activity
Chemotaxis
Phagocytosis/pinocytosis
Glucose transport and metabolism
Generation of gaseous mediators Reactive nitrogen intermediates Reactive oxygen intermediates
Enzymes Neutral proteases, elastase, lysozyme, acid hydrolases, collagenases, plasminogen activator, arginase, lipases, phosphatases 1α-hydroxylase
Plasma proteins Complement components (C1-C5, properdin) Coagulation factors (factors V, VIII, tissue factor) Finbronection
Cytokines and chemokines IL-1, IL-6, IL-10, IL-12, IL-15, IL-18, TNF-α, IFN-α, TGF-β GM-CSF, M-CSF, G-CSF IL-8, MCP-1, MIP-1α/β regulated on activation normal T expressed and secreted
Growth factors Platelet-derived growth factor, endothelial growth factor, fibroblast growth factor
Lipid mediators Eicosanoids

Macrophage activation is accomplished during infection through the release of macrophage-activating cytokines from T lymphocytes specifically sensitized to antigens of the infecting microbe (Fig. 4). The macrophage-cytokine-T lymphocyte axis plays a critical role in the development of cell-mediated immunity. In the process, IFN-γ produced by NK cells (innate immune response) and Th1 cells (adaptive immune response) is the most powerful macrophage-activating cytokine and is currently used for preventing/treating opportunistic infections in patients with immunodeficiency [11], for treating malignant melanoma [12], and for treating the decreased bone resorption of osteopetrosis, which is caused by impaired function of osteoclasts (bone macrophages) [13].

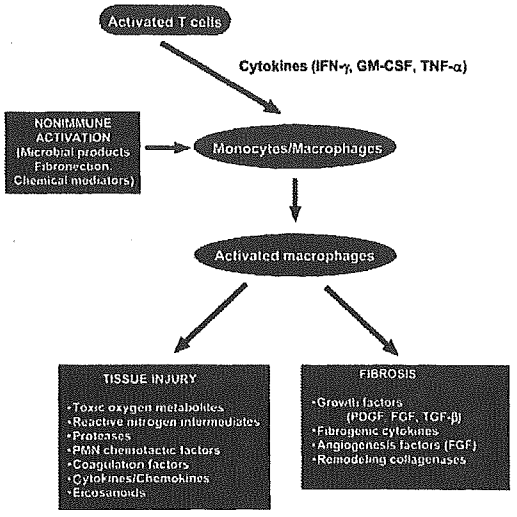

Fig. (4). Two stimuli for macrophage activation. The products by activated macrophages mediate tissue injury and fibrosis.

Macrophages exposed to bacterial LPS and/or other inflammatory stimuli release TNF-α, which itself can activate macrophages. As macrophages become activated, they express greater numbers of TNF-α receptors. Macrophages at sites of inflammation have the potential to activate themselves and express augmented function more rapidly than through the development of cell-mediated immunity, which requires recruitment of antigen-specific Th1 lymphocytes. Following surface and endocytic stimulation, macrophages can secrete a wide range of products. These include enzymes involved in antimicrobial resistance, proteinases, eicosanoids/arachidonate metabolites that contribute to inflammation and tissue repair, cytokines such as IL-1 and TNF-α that modulate the activities of leukocytes and endothelial cells, and RNIs/ROIs in host defense. Ligation of specific receptors induces various signaling pathways and is able to alter gene expression in macrophages. Transcription factors, including NF-κB [14] and PU.1 [15] families contribute to macrophage-restricted or activation dependent changes of gene expression. Expression of products depends further on translational regulation, post-translational modification such as proteolytic processing, and expression of inhibitory cytokines, IL-10 and transforming growth factor (TGF)-ß, which are produced by macrophages, fibroblasts, and T lymphocytes. Macrophage products are labile and act close to the cell surface, and overproduction results in tissue catabolism/damage and systemic effects associated with widespread infection or chronic inflammation, often as a result of an immunologically mediated disease process.

RESOLUTION OF INFLAMMATION

Inflammation is the physiologic response to damaging influences, but when allowed to continue unopposed, the subsequent cascade of events can lead to serious host injury. Inhibition of inflammation by removal or deactivation of mediators and inflammatory effector cells permits the host to repair damages tissues (Fig. 5). Although the precise mechanisms controlling the switch form proinflammatory pathways to anti-inflammatory are not fully clarified, components of resolution include a cellular response (apoptosis), formation of soluble mediators (such as anti-inflammatory cytokines and antioxidants), and production of direct effectors (such as protease inhibitors). Importantly, macrophages play a key role in the down-regulation process.

Apoptosis

Apoptosis is a conserved "program" in eukaryotic cells that leads to cell death and marks their surfaces for rapid removal by phagocytes [16]. The process fails to induce an inflammatory response, although cell death by necrosis results in the release of intracellular contents into the microenvironment provokes inflammation. Apoptosis is the normal process by which inflammatory cells are removed from healing sites. Defective apoptosis or persistence of apoptotic cells that escape clearance may contribute to chronic inflammation and autoimmune disease. Commitment to apoptosis can be provoked by various factors, including ROIs in the microenvironment and signals from death receptor pathways, FasL/Fas. Removal of apoptotic bodies and/or the remnants of packaged apoptotic cells is rapid and can be achieved by macrophages, dendritic cells, fibroblasts, epithelia, endothelia, and myocytes. The surface receptors used in recognition and engulfment of apoptotic cells include integrins, lectins, scavenger receptors, ATP-binding cassette transporter, LPS receptor, CD14, and complement receptors CR3 and CR4, although some of these molecules can be used in both proinflammatory and apoptotic pathways, the diversity may be based on distinct ligands and accessory molecules. Apoptotic cells exhibit a series of apoptotic cell associated molecular patterns distinct from PAMPs.

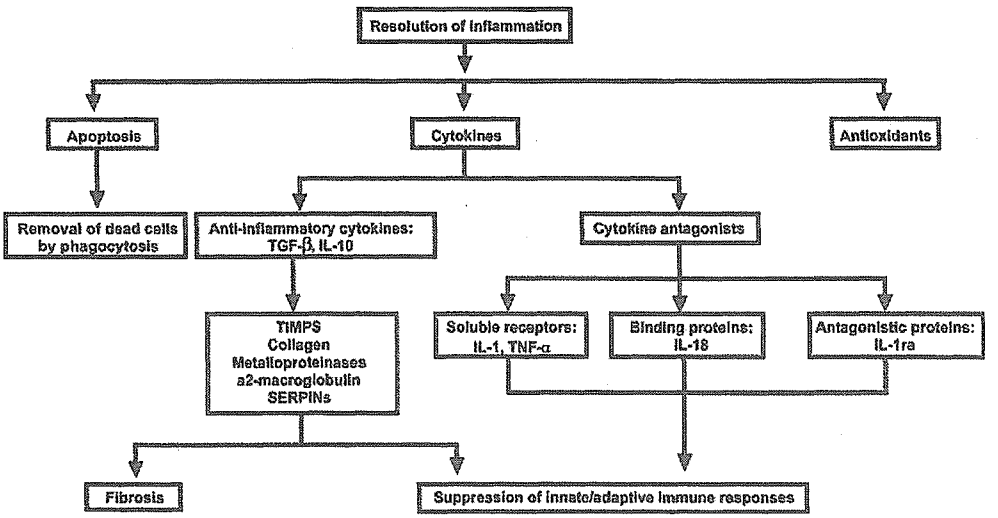

Fig. (5). Resolution of inflammation. Multiple endogenous pathways participate in the resolution.

Soluble Mediators

Antiinflammatory Cytokines

There are cytokines that initiate and induce the inflammatory response. By contrast, an array of cytokines, such as TGF-ß, IL-4, and IL-10, acts as down-regulators in the inflammatory response. The anti-inflammatory cytokines, TGF-ß and IL-10, are produced by macrophages, fibroblasts, and T lymphocytes, although IL-4 is produced by NKT and Th2 cells. TGF-ß is a potent stimulator of mesenchymal cell proliferation, which is essential for wound healing. TGF-ß suppresses collagenase production, increase collagen deposition, and decreases metalloproteinase (MMP) activity by inducing production of the tissue inhibitors of metalloproteinases (TIMPs). Although it is obvious that wound healing is necessary, the resolution of inflammation is abnormal in diseases that fibrosis represents a major pathologic feature, including systemic sclerosis, which shows a marked diffuse fibrosis associated with high levels of TGF-ß and increased extracellular matrix production [17].

IL-4 inhibits the activation of Th1 lymphocytes, and this, in turn, decreases the production of IL-1 and TNF-α and exerts anti-inflammatory activity. IL-4 also inhibits the production of IL-6 and IL-8, but increases the expression of IL-1 receptor antagonist (IL-1ra) [18].

IL-10 modulates expression of cytokines, chemokines, soluble mediators and cell surface molecules by cells. The effects of IL-10 on cytokine production and function of macrophages are generally similar to those on monocytes. IL-10 potently inhibits production of IL-1, IL-6, IL-10 itself, IL-12, IL-18, GM-CSF, G-CSF, M-CSF, TNF-α, leukemia inhibitory factor and platelet-activating factor (PAF) by activated monocytes/macrophages. The inhibitory effects of IL-10 on production of IL-1, IL-6, and TNF-α are crucial to its anti-inflammatory activities, because these cytokines have synergistic activities on inflammatory pathways and processes, and amplify these responses by induction of secondary mediators, including chemokines, prostaglandins, and PAF. IL-10 also inhibits production of both CC (monocyte chemoattractant protein; MCP1, MCP5, macrophage inflammatory protein; MIP-1α/ß, MIP-3α/ß, regulated on activation normal T expressed and secreted; RANTES) and CXC (IL-8, interferon-inducible protein; IP-10, MIP-2, GRO-α) chemokines by activated macrophages. IL-10 not only inhibits production of these effectors, but also enhances expression of their natural antagonists, including IL-1 receptor antagonist and soluble p55 (type 1) and p75 (type 2) TNF receptors, and it inhibits expression of IL-1 receptors by activated macrophages, indicating that IL-10 not only deactivates macrophages but also induces production of anti-inflammatory molecules. IL-10 inhibited production of prostaglandin E2 (PGE2), through down-regulation of cyclooxygenase 2 (COX-2) expression. Certain T lymphocyte-derived cytokines, including IL-4, IL-10, and IL-13, suppress the expression of matrix MMPs by cells stimulated with proinflammatory cytokines, such as IL-1 and TNF-α [19].

To regulate/limit the action of proinflammatory cytokines, it is known decoy receptors that recognize inflammatory cytokines with high affinity and specificity [20]. These include IL-1 and TNF-α receptors, and are structurally incapable of signaling or presenting the agonist. In rheumatoid arthritis, insufficient production of the inhibitors and may contribute to disease, and administration of exogenous antagonist has therapeutic benefit. The activity of IL-1 is regulated by two different mechanisms involving an IL-1 decoy receptor and a natural receptor antagonist protein, IL-1ra that binds to functional IL-1 receptors and competes with IL-1. IL-1ra does not transduce a signal to the cell and can block the biologic function of IL-1. Soluble cytokine receptors (eg. TNF-α receptors) can be released from cells after proteolytic cleavage of the transmembrane domain to remove the cytokine from the environment. IL-18 binding proteins capture and neutralize IL-18 activity.

Suppressors of Cytokine Signaling Proteins

The suppressor of cytokine signaling (SOCS) family of cytoplasmic proteins functions as a negative feedback loop to attenuate signal transduction from cytokines [21]. The phagocytes of the innate immune system are regulated by cytokines that are controlled by SOCS proteins, including IL-12 and interferons.

Eicosanids

COX-2 induced by inflammatory mediators contributes to the early/induction phase of inflammatory response, although COX-2 plays a role in the late/resolution [22]. In the process, cyclopentenone prostaglandins are formed and may be anti-inflammatory by inhibition of proinflammatory gene transcription. Cyclopentenone prostaglandins bind to peroxisome proliferator-activated receptor γ (PPARγ), which is present in monocytes/macrophages and inhibits the activation of macrophages and the production of proinflammatory cytokines (IL-1, IL-6, and TNF-α) by the suppression of AP-1 and STAT transcriptional pathways. Because cyclopentenone prostaglandins suppress inhibitor of NF-κB kinase (IKK)-catalyzed phosphorylation that is an essential step in the signal-induced activation of NF-κB, and consequently they inhibit NF-κB activation.

Inhibitors of Direct Inhibitors

Antioxidants

In the process of inflammation, ROIs are produced and contribute to host defense. Excessive production of then can also lead to tissue damage by reacting indiscriminately. To protect host cells and avoid tissue damage, an array of antioxidants exists. These include antioxidant enzymes, chain-breaking antioxidants, and metal-binding proteins [23].

The antioxidant enzymes include catalase and superoxide dismutase. Catalase is a peroxisomal enzyme that catalyzes the conversion of hydrogen peroxide to water and oxygen. Superoxide dismutases exhibit the dismutation of superoxide radicals to form dioxygen and hydrogen peroxide. Because ROIs are the product of activated macrophages, antioxidant enzymes indirectly inhibit the macrophage function.

Interactions of ROIs with surrounding molecules generate secondary radical species in a self-propagating chain reaction. Chain-breaking antioxidants are small molecules that receive or donate an electron and thus form a stable by-product with a radical. These antioxidants can be divided into aqueous (vitamin C, albumin, and reduced glutathione) and lipid (vitamin E, carotenoids, and flavonoids) substances. Metal-binding proteins function as antioxidants by sequestering cationic iron and copper and consequently inhibiting hydroxyl radical propagation.

Protease Inhibitors

Tissue destruction mediated by proteases is a feature of inflammation. Mechanisms to protect the host and prevent uncontrolled tissue damage using protease inhibitors have developed in the process of repair/resolution. Protease inhibitors regulate the function of endogenous proteases and reduce tissue damage. These are categorized into α2-macroglobulins and active site inhibitors. α2-macroglobulins act by covalently linking the protease to the α2-macroglobulin chain and inhibiting binding to substrates. As the active site inhibitors, inhibitors of serine proteases (SERPINs) are the most abundant and play a role in regulation of blood clot regulation and inflammation. In addition to inactivation by protease inhibitors, serine proteases are inactivated by oxidation.

Inhibition of MMP functions can be induced during the repairing/resolution phase of inflammation. A family of TIMPs suppresses most of MMPs [24]. The TIMPs bind to activated MMPs and irreversibly block their catalytic sites. Although IL-1 and TNF-α induce MMPs, TGF-ß and certain growth factors suppress MMPs and increase TIMPs and production of matrix proteins. When proinflammatory cytokines predominate, the balance favors matrix destruction. By contrast, production of matrix protein increases and MMPs are inhibited by TIMPs in the presence of inhibitors of proinflammatory cytokines and growth factors.

THERAPEUTIC INTERVENTIONS

Development of an effective inflammatory response can play an important role in the host defense. But the response can sometimes be detrimental, for example allergies, autoimmune diseases, and microbial infections may be initiate chronic inflammatory response. It is available to therapeutic approach for reducing long-term inflammatory responses.

Glucocorticosteroids

The initial subcellular events are triggered by glucocorticoid binding to cytoplasmic receptor, glucocorticoid receptor (GR). Glucocorticoids play a role in anti-inflammation/immunosuppression through their inhibition of NF-κB, which is a major factor involved in the regulation of cytokine expression [25]. NF-κB is normally located in the cytoplasm associated with the inhibitor protein IκB. There have been two mechanisms involving GR-mediated suppression of NF-κB. One is that glucocorticoids *via* GR induces the expression of IκB that then sequesters NF-κB in the cytoplasm and prevent it from translocating to the nucleus and inducing gene activation. The suppression mechanism of NF-κB by GR may be limited to certain cell types, particularly monocytes/macrophages and lymphocytes. On the other hand, there is a physical interaction or cross-talk between NF-κB and GR that prevents gene expression.

Glucocorticoids modulate cytokine expression, adhesion molecule expression and cell trafficking, expression of chemokines, and production of inflammatory mediators. Glucocorticoids suppress the expression of proinflammatory cytokines, including IL-1, IL-2, IL-6, IL-8, IL-11, IL-12, TNF-α, IFN-γ, and GM-CSF, whereas upregulating anti-inflammatory cytokines, IL-4 and IL-10 by high doses of glucocorticoids. They reduce the trafficking of leukocytes to the site of inflammation. This is mediated by the down-regulation of protein molecules involved in the attraction and adhesion of leukocytes into the site. They inhibit the expression of intercellular adhesion molecule 1 (ICAM-1), E-selection (ELAM-1), and vascular cell adhesion molecule 1 (VCAM-1). Glucocorticoids down-regulate the expression of CXC (IL-8, GRO-α) and CC (MCP1-3, RANTES, eotaxin). Glucocorticoids also suppress the production of inflammatory mediators such as nitric oxide by the inhibition of nitric oxide synthase and prostaglandins by the inhibition of phospholipase A2 and COX-2. Glucocorticoids interfere with the protective and defense mechanisms of activated macrophages and induce apoptosis in monocytes/macrophages. Consequently glucocorticoids reduce the number of circulating monocytes, and thus decrease synthesis of macrophage-derived inflammatory enzymes, including collagenases and elastases. They induce a shift from a Th1 to a Th2 pattern of adaptive immunity, because glucocorticoids down-regulate Th1 cytokines and thus result in the dominant expression of Th2 cytokines. IL-12, an initiation cytokine of Th1 response produced mainly by macrophages and dendritic cells, is down-regulated *via* inhibition of STAT4 phosphorylation in the signaling cascade downstream of IL-12.

TNF-α Antagonists

TNF-α, a proinflammatory cytokine that is released by activated monocytes/macrophages and T lymphocytes, promotes inflammatory responses. It binds two receptors, type 1 and type 2 TNF-α receptors. In addition, the biologic activity of TNF-α can be attenuated by soluble TNF-α receptors. TNF-α-based strategies are being explored for the treatment of inflammatory diseases. These include the neutralization of TNF-α by soluble receptors (etanercept) or monoclonal antibodies (adalimumab and infliximab). TNF-α antagonists are currently used for the treatment of rheumatoid arthritis, Crohn's disease, psoriasis, ankylosing spondylitis, juvenile arthritis, Still's disease, uveitis, and vasculitis [20]. It has been found the adverse effects of therapy with TNF-α, including infections, cancer, vasculitis, lupus-like-autoimmune disease, multiple sclerosis-like disorders, aplastic anemia and lymphoma., although the relationship between anti-TNF-α therapy and these adverse events are unknown.

IL-1ra

IL-1, produced by monocytes/macrophages, has proinflammatory activities. The action is down-regulated by IL-1ra, a natural inhibitor that competes for the binding to IL-1 receptors. Anakinra is a recombinant form of human IL-1ra that targets the type 1 IL-1 receptors and is used for treatment of rheumatoid arthritis [26]. Similar to the situation with TNF-α antagonists, the risk of infection appears to be increased.

IL-6 Antagonist

Humanized antibody against IL-6 receptor has shown that inhibition of IL-6 significantly improves the signs and symptoms of RA and normalized the acute-phase reactants [27].

CONCLUSIONS

The inflammatory process is usually tightly regulated, involving both signals that initiate and maintain inflammation and signals that shut the process down. An imbalance between the two signals leaves inflammation unchecked, resulting in cellular and tissue damage. Because macrophages produce a wide range of biologically active molecules participated in both beneficial and detrimental outcomes in inflammation, therapeutic interventions targeted macrophages and their products may open new avenues for controlling inflammatory diseases.

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結核における肉芽腫炎症と宿主防御の統御

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【要旨】
世界で約二〇億人が結核菌に既感染、毎年八八〇万人が結核を発病、二〇〇万人が死亡している。結核菌-宿主関係として、①宿主防御機構からの逸脱や、②遅延型過敏反応の誘導、その結果、感染から発病に至る長期の潜伏期間や組織破壊を伴う肉芽腫炎症が特徴的である。

◆キーワード
結核菌-宿主関係
細胞性免疫
潜伏感染
再興感染症
サイトカイン

はじめに

世界の年間総死亡は約五七〇〇万人で、その主な内訳として、循環器疾患(虚血性心疾患や脳血管障害など)一六七〇万人、感染症一四九〇万人、悪性新生物七一〇万人であり、感染症は現在でも全世界の総死亡の四分の一強を占め、人類に大きな健康被害を招来している。感染症による死亡(一四九〇万人/年)の主要な原因として、急性呼吸器感染症(肺炎など)三九六万人、後天性免疫不全症候群(AIDS,結核の合併を含む)二七七万人、下痢性疾患一八〇万人、結核一五六万人、マラリア一二七万人や麻疹六一万人などがある[1]。

全世界では、約二〇億人(全人口の三分の一)が結核菌(*Mycobacterium tuberculosis*)に既感染、毎年八八〇万人が結核を発病、二〇〇万人(AIDS合併を含む)が死亡し、有病者は二二〇〇万人である[2]。今後一〇年間、少なくとも、八〇〇〇万人が発病、二〇〇〇万人が死亡することが推定されている。日本(二〇〇三年)では年間三万二〇〇〇人(罹患率人口一〇万対:二四・八)が結核を発病し、二三〇〇人(死亡率:一・八)が死亡し、有病者は三万人(有病率:二三・三)であり、結核は単一病原体による感染症として、世界最大である。

結核は結核菌-宿主関係から成立し、その結果、病変形成と宿主防御を招来する。結核の代表的な病変は病理形態学的に〝肉芽腫炎症〟であり、宿主防御として九〇%の結核菌感染者が防御機序により発病を回避している。しかし、結核における〝病変形成と宿主防御〟の詳細な細胞・分子機序は未解明である。

本稿では、結核における〝病変形成と宿主防御〟の細胞・分子機序を概説する。

結核菌の概要

結核菌(*M. tuberculosis*)の生物学的特徴として、①細胞内寄生性、②脂質成分に富む細胞壁、③好気性、④遅発育性、⑤空気(飛沫核)感染、⑥慢性炎症、および⑦遺伝

子の解読などがある（表1）。

結核菌など抗酸菌は、基本的に外毒素や内毒素非産生性であるが、例外的に*M. ulcerans*（西アフリカ諸国やオーストラリアで猛威を振るっているBuruli潰瘍の原因菌）が外毒素（mycolactone, 別名polyketide toxin, 宿主組織に壊死を惹起する）を産生する。

炎症病変や組織障害は、結核菌に対する感染免疫応答過程で宿主から産生されるサイトカインをはじめとする生理活性物質に依存している[3]。

表1　結核菌の特徴

細胞内寄生性	桿菌（0.2～0.6×1～10μm），宿主細胞，特にマクロファージ内で抗菌機構から逃れて増殖
細胞壁	脂質成分が豊富なため，疎水性であり，化学物質にも安定，グラム染色に難染色性，抗酸性
好気性	酸素分圧の高い臓器（肺など）で増殖し，病変を形成
遅発育性	至適温度：37℃，倍加時間：約12～15時間，培養集落形成に4～8週間
感染形式	飛沫核／空気感染
病原性	慢性炎症，肉芽腫，乾酪壊死，空洞形成，線維化
遺伝子	全ゲノム（約4.41 Mb）の解読

結核菌の細胞壁は長鎖脂肪酸（ミコール酸）に富んでおり、グラム染色では難染色性を示す。そのため、抗酸性（Ziehl-Neelsen, Kinyoun）染色や蛍光染色が用いられる。分裂倍加時間は約一二～一五時間の遅発育菌であり、感染伝播は飛沫核（空気）感染による。

宿主防御機構では、マクロファージ-サイトカイン-T細胞応答系、すなわち細胞性免疫が役割を演じ、細胞内殺菌物質として、ガス状物質（反応性酸素化合物質や反応性窒素化合物質）が寄与している。その結果、結核菌感染者の約一〇%が一生涯において結核を発病する。病変は慢性炎症、肉芽腫、乾酪壊死、空洞形成や線維化などが特徴的である。

M. tuberculosis H37Rvの全ゲノム塩基配列が解明された[4]。今後、遺伝子解析を基盤とした科学的戦略が推進され、分子／遺伝子標的を視点とした新規診断法、抗結核薬の開発、薬剤耐性獲得機構の解明や新規ワクチン開発が展開されるであろう。

結核病態の細胞・分子機構

結核菌の病原性として、(1)宿主防御機構から逸脱した細胞内生存および増殖（細胞内寄生病原体）や、(2)遅延型過敏反応（細胞性免疫応答の負の側面）の誘導が特徴的であり、その結果、結核菌感染から発病に至る長期の潜伏期間や組織破壊を伴う肉芽腫炎症が発現する[5]。

結核菌の細胞内寄生性と潜伏感染

肺結核患者（特に、喀痰塗抹陽性）から曝露された約三〇%に結核菌感染が成立し、感染者の約一〇%が一生涯において結核を発病する。有効な感染防御応答により、九〇%の感染者は結核菌を封じ込め、発病を回避している。宿主に気道を介し侵入した結核菌はマクロファージに貪食され、マクロファージ食胞体（ファゴソーム）と水解小体（リソソーム）の融合を阻害することにより、酸性化されず、食胞体内で生存し続ける（細胞内寄生病原体）。

結核菌は宿主内で潜伏感染し、潜伏感染した結核菌は宿主免疫機構の破綻（老化、免疫抑制薬／副腎皮質ステロイド薬投与、栄養障害、HIV感染／AIDSなど）により、発育・増殖を再開し、結核を発病する（内因性再燃）。人類の約三分の一が結核菌に潜伏感染している事実を考慮すると、潜伏感染機序を解明することは新規抗結核薬やワクチン開発を促進し、その

結果、結核制圧に寄与するであろう[6]。

潜伏感染した結核菌の特性として、(1)定常期に発現するσ因子(*sigF*遺伝子、対数増殖期には未発現)や(2)糖代謝から脂質代謝への変換が知られている。*sigF*欠損結核菌は肉芽腫内で生存不能であり、肉芽腫内生存結核菌はglyoxylate shunt(脂肪酸から糖代謝への変換酵素系、哺乳動物の冬眠におけるエネルギー代謝変換に類似)を亢進させている[7]。

また、潜伏感染結核菌はmycobacterial DNA-binding protein 1(MDP1, 分子量約二八kDa)を多量に発現している。MDP1は抗酸菌体内や表層に存在する抗酸菌特異的蛋白質である。機能的に、細胞質内MDP1は核酸結合性を示し、転写および翻訳阻害活性を有し、そのため休眠機構、さらに潜伏感染に関与していることが示唆されている。

他方、菌表層MDP1の生物学的意義は不明であった。しかし最近、MDP1が宿主細胞表面に存在するムコ多糖(グリコサアミノグリカン)、特にヒアルロン酸に結合し、抗酸菌の接着/侵入に関与することが判明した[8]。また、bacillus Calmette-Guérin(BCG)や結核菌の宿主細胞接着が、ヒアルロン酸やMDP1の細胞接着阻害物質であるDNAや抗MDP1抗体で抑制された。

これらの結果は、抗酸菌の上皮細胞への接着/侵入におけるヒアルロン酸の重要性を示し、MDP1が菌の接着/侵入を促す接着分子であることを示唆している。実際、ヒアルロン酸や抗MDP1抗体をマウス気道感染モデルに投与した結果、感染菌数が著しく減少した。この成果は、結核菌MDP1が治療標的として有望な候補になる可能性を示している。

結核性肉芽腫炎症の機序

肉芽腫病変は病理形態学的に「巣状炎症であって、主としてマクロファージ、マクロファージが活性化または分化した細胞と考えられる類上皮細胞や多核巨細胞などが集積あるいは増殖して惹起された病変」である。発症機序により、過敏性(免疫性)と異物性肉芽腫に分類される。過敏性肉芽腫は免疫応答(特に、T細胞性)により惹起され、異物性肉芽腫は免疫応答が関与せずに、惹起物質が直接炎症性細胞を刺激することにより誘導される(図1)。

抗酸菌性肉芽腫は、抗酸菌に対する細胞性免疫発現によって増強されるが、細胞性免疫が発現しない未免疫マウスや無胸腺ヌードマウスでも肉芽腫が形成されることから、過敏性および異物性因子の複合した混合性肉芽腫病変である[9]~[11]。

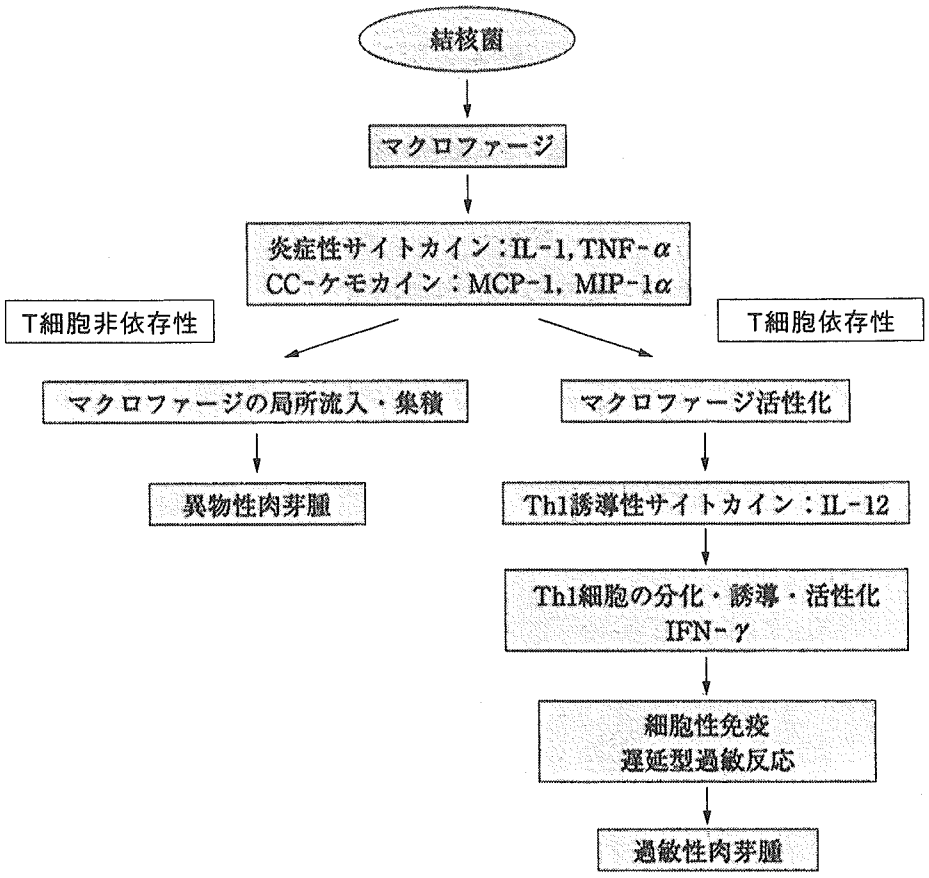
肉芽腫病変部にマクロファージやT細胞浸潤が認められ、これらの細胞は炎症や免疫の制御に重要なサイトカインを産生している。肉芽腫炎症局所には、病変活動性に一致してインターロイキン(IL)-1, 腫瘍壊死因子(TNF)-α, マクロファージ遊走阻止因子(MIF)、マクロファージ炎症性蛋白(MIP)-1αや単球走化性蛋白(MCP)-1などが存在する。さらに、これらのサイトカインを不溶化担体に結合させて実験動物や培養系に投与/添加することによって肉芽腫が形成される。すなわち、IL-1, TNF-α, MIF, MIP-1αやMCP-1などは肉芽腫誘導性サイトカインである[10][12]~[15]。MIP-1αやMCP-1はCC-ケモカインとして単球走化活性を、MIFはマクロファージ遊走阻止活性を有し、病変部への単球の流入や集積に関与している。

IL-1やTNF-α自身は単球走化活性をほとんど示さないが、単球由来MIP-1αやMCP-1産生/誘導活性が顕著であることから、IL-1やTNF-αに認められた肉芽腫誘導活性はCC-ケモカインを介する間接的効果と考えられる。このことは、ツベルクリン皮内反応陽性ヒト末梢血単球を結核菌やツベルクリン蛋白で刺激すると最も早期(二四時間以内)にIL-1が誘導され、遅延型皮内反応が発現する四八~七二時間後にMCP-1が誘導される事実からも支持される[16][17]。

肉芽腫誘導性サイトカインの主な産生細胞はマクロファージであ

り、さらに感染病変局所に最も多く集積している細胞もマクロファージであることから、マクロファージは結核菌感染に対する宿主防御反応である肉芽腫炎症において、中心的かつ必須の役割を演じている[3) 18)]。実際、in vitro肉芽腫炎症モデルを解析した結果、マクロファージおよびマクロファージ由来サイトカインが肉芽腫の最小構成単位を形成していることが判明し、in vivo病変形成機序を支持している[15)]。

図1　結核性肉芽腫炎症の細胞・機能分子機序



マクロファージ抑制物質（IL-4, プロスタグランディンE、糖質コルチコイド）やIL-1受容体拮抗蛋白（IL-1ra）は、炎症性サイトカイン発現や活性を抑制する作用があり、肉芽腫炎症抑制物質である[19)]。反応性酸素化合物（ROIs）は単球／マクロファージ由来IL-1の産生誘導活性を有し、スーパーオキシドジスムターゼ（SOD）投与により肉芽腫炎症は抑制される[20)]。したがって、ROIsは肉芽腫炎症惹起物質であり、SODは肉芽腫炎症抑制物質である。

肉芽腫炎症と感染防御の統御

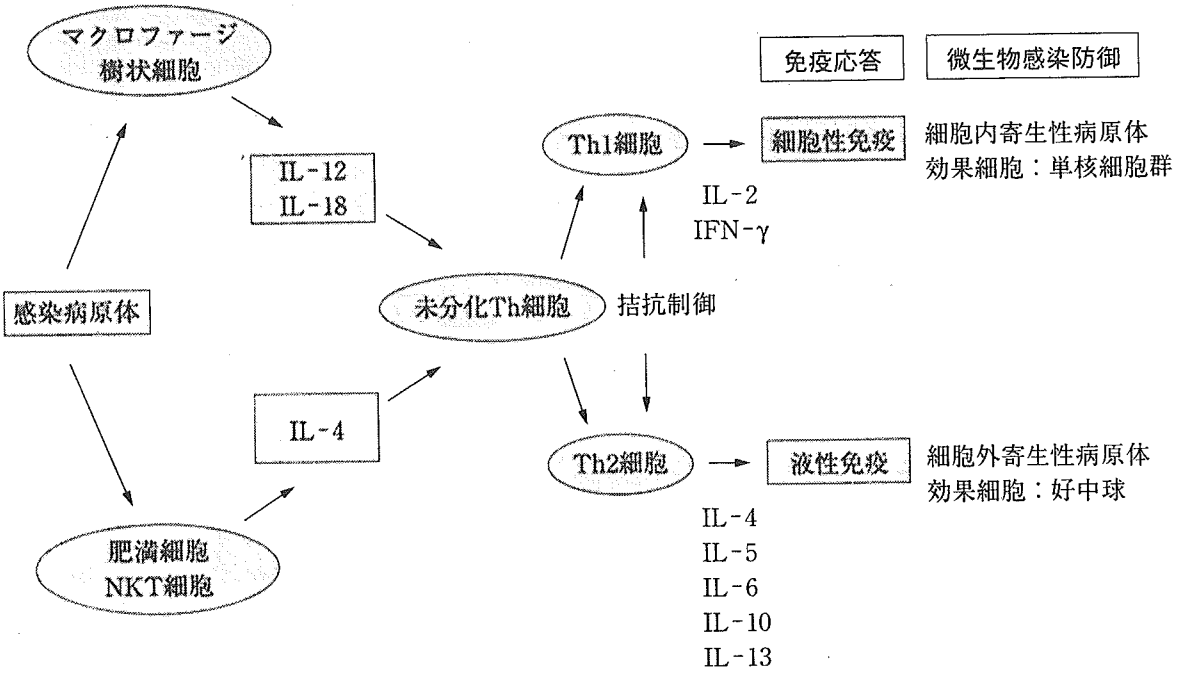
結核菌など抗酸菌は細胞内寄生病原体であり、宿主防御にマクロファージ-サイトカイン-CD4陽性1型ヘルパーT（Th1）細胞応答系、細胞性免疫が貢献している（図2）。

細胞性免疫の起動サイトカインとして、IL-12, IL-18やインターフェロン（IFN）-γがTh1細胞分化や活性化など、重要な役割を演じている[21)]。しかし、結核菌感染に対する遅延型過敏反応を含む細胞性免疫の発現は抗結核菌防御と組織傷害に貢献、すなわち功罪の二面性（諸刃の剣）を表現する[18)]。

また遺伝的因子として、ヒト第2染色体に存在する遺伝子（*NRAMP1*; natural resistance associated macrophage protein 1, 別名*SLC11A1*）が感染防御に関与し、この機能はマクロファージに表現されている[22) 23)]。抗酸菌感染部位における宿主応答はマクロファージ（類上皮細胞や多核巨細胞を含む）およびT細胞の局所集積を特徴とする肉芽腫炎症であり、その成立機序には細胞性免疫発現が関与している[3) 18) 24)]。肉芽腫炎症は遺伝的感染感受性を示す宿主に顕著である[3) 9) 25)~27)]。

すなわち、*Nramp1*遺伝子感受性を表現するマウスでは抗酸菌増殖および肉芽腫炎症増強、炎症性サイトカイン（IL-1, TNF-αや単球走化性ケモカインなど）産生亢進、防御性サイトカイン（IL-12やIL-18）産生低下が判明し、抵抗性マウスではその逆であっ

図2　微生物感染における細胞性および液性免疫応答



た。肉芽腫炎症は結核菌など宿主に侵入・感染した抗酸菌を局所に封じ込め、かつ、結核性肉芽腫の特徴である乾酪壊死は無血管領域であるため、好気性である結核菌の発育・増殖を抑制し、宿主に合目的な防御応答である。

これらの事実は、抗酸菌に対する宿主防御応答である肉芽腫炎症は、遺伝的抵抗性宿主において肉芽腫炎症を発現しなくても抗酸菌感染を制圧することが可能であり、感染防御にこの病変形成は不要であるが、感受性宿主には生存や防御のため必須であることを示している（図3）。

結核菌感染における宿主応答と細胞壁糖脂質

結核菌の脂質は乾燥菌体重量の一〇%以上、細胞壁の二〇%以上を構成し、他の一般細菌に比し、きわめて多い。事実、結核菌の全ゲノムは約四・四Mb（大腸菌四・六Mb）であり、蛋白質を規定している遺伝子は約四〇〇〇、脂肪酸代謝に関与している酵素は二五〇以上、大腸菌が五〇であることから、結核菌の脂質代謝がきわめて旺盛であることが遺伝子情報からも判明している[27]。

結核菌細胞壁の脂質として、mycolyl-arabinogalactan-peptidoglycan complex, lipoarabinomannan (LAM), lipomannan, phosphatidyl-myo-inositol, sulfolipid (SL), trehalose 6, 6'-dimycolate (TDM) /cord factor, phenolic glycolipid や lipooligosaccharides などの糖脂質が特徴的である[28]。

特に、アシル化trehalose脂質化合物であるTDM/cord factorやSLが結核菌に特徴的であり、結核菌-宿主関係、すなわち、病原性や毒性の発現に関与している。また、〝抗酸性〟に主として関与する菌体表層成分はミコール酸などの脂質成分であり、ミコール酸は天然で稀なα位に分枝鎖、β位に水酸基を持つ長鎖脂肪酸（結核菌では炭素数六〇～九〇）である。

宿主マクロファージは吸入した結核菌を貪食し、食胞体（phago-

some）を形成する。アシル化trehalose脂質化合物（SLやTDM）は食胞体と加水分解酵素を含むリソソーム（lysosome）の融合（P-L fusion）を阻止することにより、結核菌が酸性化されず、食胞体内、すなわち宿主マクロファージ内で生存を可能にしている。

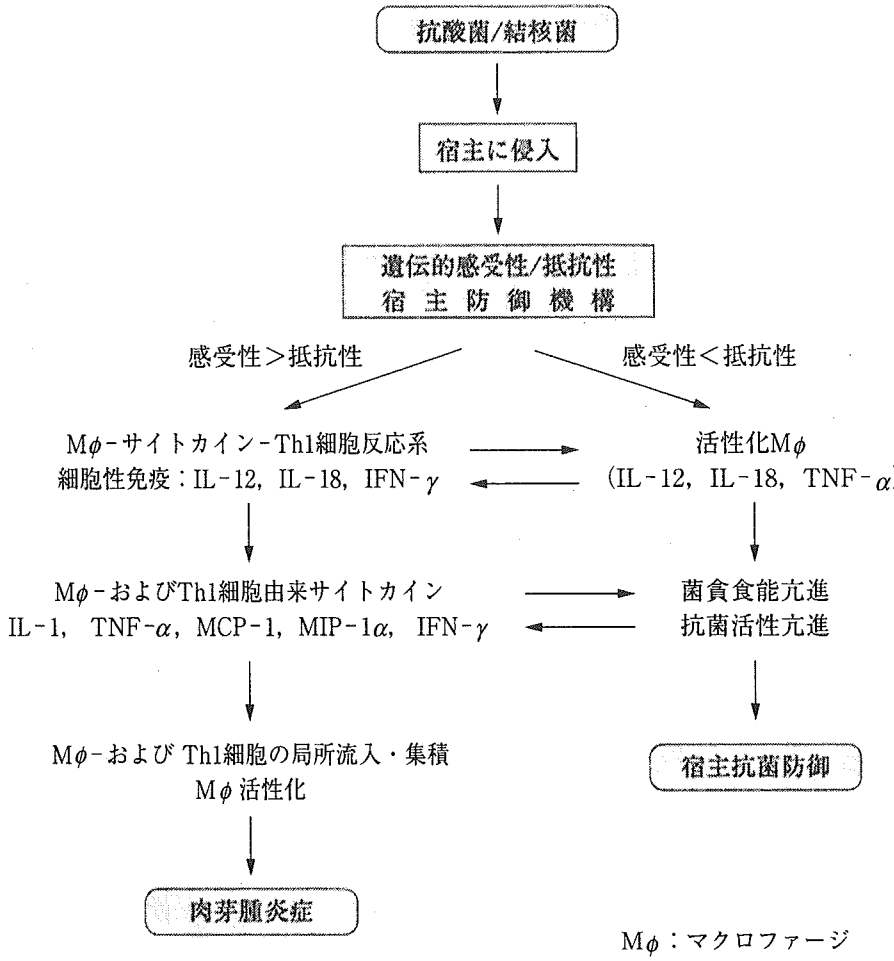
肉芽腫炎症は、発症機序により異物性（T細胞非依存性）および過敏性（T細胞依存性）に大別（図1）されるが、結核菌の病原因子と宿主応答について解析した。結核菌TDMを無胸腺ヌードマウスや未免疫マウスに投与することにより、肉芽腫を惹起できること、TDM免疫マウスではTDM誘導肉芽腫が増強されることや、遅延型過敏反応／細胞性免疫の指標である足蹠腫脹反応が誘導されることから、TDMは異物性ならびに過敏性の両機序を介して肉芽腫を形成する[9)11)]。事実、TDM誘導肉芽腫病変は多量の細胞性免疫起動性サイトカイン（IL-12やIFN-γ）を含み、活動性に伴い消長する。したがって、結核肉芽腫の発現に異物性および過敏性の両機序が複合関与しており、結核肉芽腫は混合性肉芽腫である。

肉芽腫形成には病変局所への血中単球の流入や活性化が必須であり、このため、局所のCC-ケモカイン産生や血管新生が重要な役割を演じる。TDMは血管内皮細胞増殖因子（vascular endothelial growth factor：VEGF）を誘導し、局所の血管新生に寄与している[29)]。

加えて、TDMは宿主免疫担当細胞（胸腺や脾臓）にアポトーシスを誘導し、その結果、細胞内寄生病原体の増殖や生存を困難にし、さらに胸腺内自己反応性T細胞を除去、Th1/Th2細胞の分化を制御することによって自己免疫疾患の発症を防止している可能性がある[30)]。しかし、SLはP-L fusion阻止（宿主細胞内生存）を発揮したが、炎症・免疫惹起やアポトーシス誘導活性をまったく示さず、TDMと対照的である[11)29)30)]。

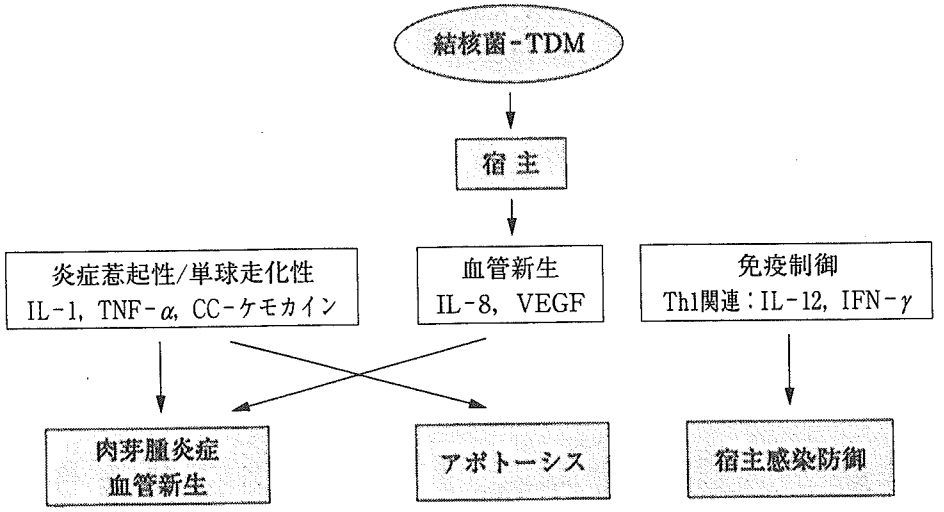
アシル化trehalose脂質化合物であるTDMは結核菌細胞壁表層に存在し、①結核菌の宿主細胞内生存、②炎症・免疫惹起物質（肉芽腫炎症、遅延型過敏反応や血管新生など）や③アポトーシス誘導活性を発揮する多機能分子（**図4**）であり、結核菌―宿主関係、すなわち結核の病態形成に重要な役割を演じている。

図3　抗酸菌／結核菌感染における宿主細胞および機能分子応答機構



Mφ：マクロファージ

図4　結核菌細胞壁TDMと宿主応答の分子機序



おわりに

結核など抗酸菌感染における宿主防御と肉芽腫炎症機構は菌および宿主側因子が関与する病原体-宿主相互関係（pathogen-host interactions）を介して成立し、抗酸菌と宿主の壮絶な生存戦争を反映している。

肉芽腫炎症は結核菌を局所に封じ込め、感染の拡大を防止し、宿主防御として有益であるが、反面、慢性炎症として自己組織破壊（乾酪壊死や空洞形成）や線維症を伴い、その結果として臓器機能不全を招来し、宿主に不利益を与える側面も有している。

宿主防御や肉芽腫炎症機構の理解は宿主抵抗性を効率的に発現するシステム（ワクチンなど）の開発も促進する[31]。しかし、感染症における宿主防御機構と病態形成は〝諸刃の剣〟、表裏一体の関係に位置している。すなわち、結核制圧において、病原体-宿主関係をよりよく理解することは制圧戦略を構築するために必須である。

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The Radioprotective 105/MD-1 Complex Links TLR2 and TLR4/MD-2 in Antibody Response to Microbial Membranes[1]

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Low-affinity IgG3 Abs to microbial membranes are important for primary immune defense against microbes, but little is known about the importance of TLRs in their production. IgG3 levels were extremely low in mice lacking radioprotective 105 (RP105), a B cell surface molecule structurally related to TLRs. RP105$^{-/-}$ B cells proliferated poorly in response to not only the TLR4 ligand LPS but also TLR2 ligand lipoproteins, both of which mediate the immunostimulatory activity of microbial membranes. RP105$^{-/-}$ mice were severely impaired in hapten-specific Ab production against LPS or lipoproteins. CD138 (syndecan-1)-positive plasma cells were detected after lipid A injection in wild-type spleen but much less in RP105$^{-/-}$ spleen. RP105 ligation in vivo induced plasma cell differentiation. RP105 expression was ~3-fold higher on marginal zone B cells than on follicular and B1 cells and was down-regulated on germinal center cells. These results demonstrate that a signal via RP105 is uniquely important for regulating TLR-dependent Ab production to microbial membranes. ***The Journal of Immunology,*** **2005, 174: 7043–7049.**

Innate immunity provides a first line of defense against microbial pathogens (1) and is dependent on a restricted set of nonvariant, germline-encoded molecules that include secreted opsonins, C-type lectins, and scavenger receptors (2). B cells can contribute to innate immunity by secreting Abs with similarities to innate immune receptors in that they are semi-invariant and reactive with both self and microbial membrane glycolipids (3). These are principally IgM and IgG3 Abs that directly bind to microbial membranes, activate complement, facilitate their phagocytosis, and enhance their immunogenicity through Ag-trapping in secondary lymphoid organs (4, 5). They are produced as natural Abs to self-antigens and microbial flora or produced in primary, T-independent (TI)[5] responses during microbial infections. B1 cells and splenic marginal zone (MZ) B cells are important for producing these protective IgM and IgG3 Abs. However, the recognition molecules on B cells that mediate these TI Ab responses have been poorly understood.

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[5] Abbreviations used in this paper: TI, T independent; MZ, marginal zone; RP105, radioprotective 105; PNA, peanut agglutinin; TNP. trinitrophenyl; HPRT, hypoxanthine-guanine phosphoribosyltransferase; GC, germinal center; TD, thymus dependent.

Microbial membranes were known to stimulate immune cells. LPS and lipoproteins were identified as principal components of the immunostimulating activity of microbial membranes. These components have turned out to be ligands for TLRs, a family of innate immune receptors for microbial products (6, 7). Heterodimers such as TLR1/TLR2 or TLR2/TLR6 mediate responses to particular membrane lipoproteins (8), whereas the TLR4/MD-2 complex is essential for LPS recognition (9, 10). LPS is a prototypical TI type 1 Ag. That is, it elicits Ab production without T cell help. Since B cells lacking TLR4 or MD-2 do not respond to LPS (10, 11), TLR4/MD-2 is required for LPS-induced Ab production. Much less is known about the importance of TLR2-mediated Ab responses to microbial lipoproteins.

B cells express another pair of TLR family proteins that are also important for LPS responses. Radioprotective 105 (RP105) forms a complex with MD-1 and can transmit powerful survival as well as proliferation signals when cross-linked by Abs (12–14). RP105$^{-/-}$ or MD-1$^{-/-}$ mice, like animals deficient in TLR4 or MD-2, are hyporesponsive to LPS (15, 16), but precise mechanisms that functionally couple the two types of complex remain unclear.

This study explored the relative importance of TLR family of receptors including TLR2, TLR4/MD-2, or RP105/MD-1 for innate Ab responses to microbial membranes. We found that RP105/MD-1 controls Ab production mediated via TLR2 and TLR4/MD-2 receptor complexes.

Materials and Methods

Reagents and mice

Lipid A from *Salmonella minnesota* and trinitrophenyl (TNP)-LPS were purchased from Sigma-Aldrich. Pam_3CSK_4, MALP-2, and their FITC conjugates were purchased from EMC Microcollections. CpG (TCCATGAC GTTCCTGATGCT) was purchased from Hokkaido System Science. TLR7 ligand loxoribine (7-allyl-7,8-dihydro-8-oxo-guanosine) (17) was purchased from InvivoGen. The following Abs for flow cytometry were purchased from eBioscience: FITC-conjugated B220, biotinylated CD86, biotinylated TLR2, biotinylated RP105, and biotinylated TLR4/MD-2. Biotinylated CD138, FITC-CD21, PE-CD23, PerCP-B220, allophycocyanin-streptavidin and PerCP-streptavidin were purchased from BD Pharmingen. FITC-IgM was purchased from Sigma-Aldrich.

0022-1767/05/$02.00

C57BL/6 and BALB/c mice were purchased from Japan SLC. Mutant mice were maintained in the animal facility at the Institute of Medical Science, University of Tokyo.

Cell staining and flow cytometry

Single cell suspensions were incubated at 2×10^5 cells/100 μl on ice for 15 min with primary Ab diluted in staining buffer (PBS containing 2.5% FCS and 0.01% NaN_3). Cells were washed in staining buffer, and incubated with R-PE-conjugated streptavidin (Southern Biotechnology Associates) for 15 min. Flow cytometry analysis was conducted on a FACSCalibur System (BD Biosciences).

B cell proliferation in vitro

Splenic B cells were purified by negative selection with CD43 microbeads on an autoMACS column (Miltenyi Biotec). B cell purity was >95%, as judged by flow cytometry analyses (data not shown). The wild-type and mutant B cells were incubated at 2×10^5/well in 96-well flat-bottom plates with TLR ligands. After 3 days culture, B cells were pulsed with 0.2 μCi/well [^{3}H]thymidine (Amersham Biosciences) for 6 h before harvesting onto glass filter. Incorporation of [^{3}H]thymidine was measured by tritium-sensitive avalanche gas ionization methods using a Matrix 96 direct beta counter (Packard Instrument).

Section staining

Ten days after i.p. injection of 100 μl of a 10% SRBC suspension in PBS, the mouse spleens were harvested, and frozen sections were stained as previously described (18) with anti-RP105 mAb followed by Alexa-594-conjugated anti-rat IgG (Invitrogen Life Technologies) and biotinylated peanut agglutinin (PNA; Vector Laboratories) followed by streptavidin FITC (eBioscience).

Determination of serum immunoglobulins and Ab responses in vivo

Wild-type and RP105$^{-/-}$ mice were immunized i.p. with TNP-LPS (50 μg/mice), FITC-Pam_3CSK_4 (50 μg/mice), or FITC-MALP-2 (50 μg/mice). The serum concentration of hapten-specific Abs at different time points was measured by hapten-specific ELISA. ELISA was performed by coating plastic plates with TNP-BSA or FITC-BSA (10 μg/ml), and serial serum dilutions were applied onto the plate. Bound Abs were detected by goat Abs to IgG1, IgG2a, IgG2b, IgG3, IgM, or IgA (Southern Biotechnology Associates). To determine serum Ig titers, goat anti-Ig Ab was coated instead of haptenated BSA. For Abs to bacteria, heat-killed bacteria were coated.

Bone marrow-derived macrophages and TNF-α ELISA

Bone marrow cells were plated in 10-cm bacteriological plastic plates with 10% FCS-RPMI 1640 supplemented with 100 ng/ml recombinant murine M-CSF (Genzyme Techne). At day 7, adherent cells were harvested by scraper, plated at 1×10^5 cells/ml in 96-well plates, and cultured with or without TLR ligands in 10% FCS-RPMI 1640 without M-CSF for 24 h. TNF-α in culture supernatants were determined using ELISA kits (BioSource International).

Preparation of mRNA and RT-PCR

Total RNA was extracted using RNeasy kit (Qiagen) according to the manufacturer's instruction. cDNA was synthesized with SuperScript first-strand cDNA synthesis system for RT-PCR (Invitrogen Life Technologies). PCR of the cDNA was performed with Blimp-1 or hypoxanthine-guanine phosphoribosyltransferase (HPRT)-specific primers. The PCR products were separated by electrophoresis on a 1.5% agarose gel and visualized by ethidium bromide staining. The primer sequence was as follows: TLR4 sense, TGGCCTCTCTAGAAAGCTTC; TLR4 antisense, TGCAGAAACATTCGCCAAGC; MD-2 sense, TTTTCGACGCTGCTTTCTCC; MD-2 anti-sense, TCAGTATCCCCAGCAATAGC; mouse Blimp-1 sense, TGACGGGGGTACTTCTGTTC; Blimp-1 anti-sense, TGGGGACACTCTTTGGGTAG; HPRT sense, TGATGAACCAGGTTATGACCTAG; HPRT anti-sense, CCAGCAAGCTTGCAACCTTAACC. The PCR conditions were 94°C denaturing for 30 s, 55°C annealing for 30 s, and 72°C elongation for 30 s for a total of 27 cycles (Fig. 6), 30 cycles (Blimp-1), or 25 cycles (HPRT in Fig. 7).

Results

Serum Ig levels are depressed in some TLR mutant and MyD88$^{-/-}$ mice

To explore a role for TLRs in serum Ab production, we first studied serum Ig levels in mice lacking MyD88, TLR2, TLR4, or RP105. MyD88$^{-/-}$ mice had higher IgG1 but lower IgG2a and IgG3 levels than wild-type mice (Fig. 1*a*). The immune system of MyD88$^{-/-}$ mice is skewed toward Th2 responses due to a lack of MyD88-dependent Th1 polarization (19, 20), and this could account for their high titers of serum IgG1 and their low titers of IgG2a. However, Th2 polarization would not explain the low IgG3 titers.

In contrast to MyD88$^{-/-}$ mice, we found no significant alterations in either IgG1 or IgG2a in TLR2$^{-/-}$ or TLR4$^{-/-}$ mutant mice (Fig. 1, *b* and *c*). Modest reductions in serum IgG3 were observed in TLR4$^{-/-}$ ($p = 0.0016$) mice. RP105$^{-/-}$ mice were severely impaired with respect to serum IgG3 on two genetic backgrounds ($p = 0.0015$ for C57BL/6 and $p = 0.000037$ for BALB/c

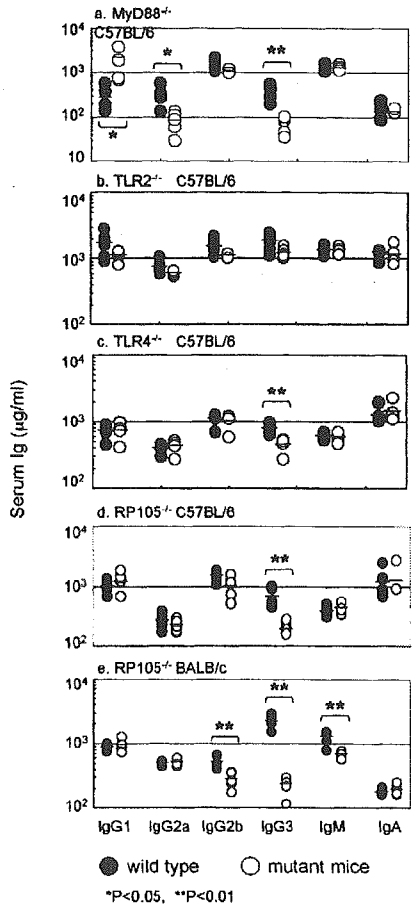

FIGURE 1. Low serum IgG3 in MyD88$^{-/-}$, TLR4$^{-/-}$, and RP105$^{-/-}$ mice. Sera were collected from MyD88$^{-/-}$, TLR2$^{-/-}$ (12 wk old), TLR4$^{-/-}$, RP105$^{-/-}$ (11 wk old for C57BL/6, 12 wk old for BALB/c) mice as indicated in the figure. The genetic background of these mice were C57BL/6. We used RP105$^{-/-}$ mice back-crossed on C57BL/6 or BALB/c backgrounds. Ig titers were determined by ELISA (see *Materials and Methods*). Black bars indicate the averages from each group (5–6 mice). Results with age-matched wild-type mice and mutant mice were shown by closed and open circles, respectively (*, $p < 0.05$, **, $p < 0.01$ by Student's *t* test). Serum Ig titers in MyD88$^{-/-}$ mice and their wild-type controls differed somewhat from the other wild-type and mutant mice, perhaps because the animal facility for MyD88$^{-/-}$ mice in Osaka University was different from the one used for the rest of the mutant mice (Tokyo University).