Etiology of Sarcoidosis: the Role of *Propionibacterium acnes*

Yoshinobu Eishi¹, Ikuo Ishige¹, Yuki Ishige¹, Tetsuo Yamada¹, Junko Minami¹, Satoshi Ikeda¹ and Morio Koike¹

¹Department of Human Pathology, Tokyo Medical and Dental University, Tokyo 113–8519, Japan

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Sarcoidosis, of unknown etiology, may result from exposure of a genetically susceptible subject to a specific environmental agent(s), possibly an infectious one, although none has been identified. *Propionibacterium acnes* is so far the only bacterium to be isolated from sarcoid lesions. Genomes of *P. acnes* have been detected in large numbers in sarcoid lymph nodes by the quantitative polymerase chain reaction. By *in situ* hybridization, *P. acnes* genomes were found in sarcoid lymph nodes in and around sarcoid granulomas. These results point to an etiological link between *P. acnes* and some cases of sarcoidosis. Host factors may be more critical than agent factors in the etiology of sarcoidosis, as already suggested from the phenomenon of the Kveim test, in which a suspension of sarcoid tissues injected intracutaneously causes sarcoid granulomas in patients with sarcoidosis but not in healthy people or patients with other diseases. A recombinant trigger-factor protein, RP35, from *P. acnes* causes a cellular immune response in some patients with sarcoidosis, but not in subjects without sarcoidosis. RP35 caused pulmonary granulomas in mice sensitized with the protein and adjuvant. Sarcoid granulomas may form during hypersensitivity to antigens of *P. acnes* indigenous to or proliferating in the affected organ.

Key words: sarcoidosis, *Propionibacterium acnes*, *in situ* hybridization, quantitative PCR, trigger factor

I. Introduction

More than 100 years have passed since the first description of sarcoid lesions by Jonathon Hutchinson, but the cause of this systemic granulomatous disease is still unknown. Sarcoidosis seems to result from exposure of a genetically susceptible subject to a specific environmental agent(s) [18]. Although an infectious agent is suspected, none has been identified. In this article, recent reports on etiology of sarcoidosis are reviewed. Evidence for the role of *Propionibacterium acnes* is accumulating in a series of studies from Japan. For one thing, *in situ* hybridization (ISH) that used catalyzed reporter deposition for signal amplification gave results suggesting that *P. acnes* may be implicated. Mechanisms of granuloma formation by this indigenous bacterium in patients with sarcoidosis are proposed here on the basis of our results obtained by molecular and experimental approaches, and a new concept of infectious disease is introduced in connection to endogenous infection caused by indigenous bacteria.

II. Putative Causative Agents of Sarcoidosis

There are many infectious agents that cause granulomas resembling those of sarcoidosis, including mycobacteria, herpesviruses, *Histoplasma capsulatum*, *Treponema pallidum*, *Sporothrix schenckii*, *Coccidioides immitis*, *Schistosoma japonicum*, *Listeria monocytogenes*, *Rhodo-
coccus species, and the agent of Whipple’s disease. Sarcoi-
dosis has a worldwide distribution, so the putative agent(s)
that causes sarcoidosis also must be widely distributed.

Similarities of clinical, histologic, and immunologic
features between sarcoidosis and tuberculosis have led some
to think that sarcoidosis may be an atypical kind of tuber-
culosis; however, Mycobacterium tuberculosis has not been
isolated in culture from sarcoid lesions. Using the poly-
merase chain reaction (PCR), some investigators in Europe
detected mycobacterial DNA in samples of affected tissue
from patients with sarcoidosis [10, 32, 34], but others did not
[2, 33, 41]. Other evidence of mycobacterial involvement
in sarcoidosis was found by Graham et al., who obtained
spheroplasts (bacteria with defective cell walls) that reverted
to acid-fast bacteria during culture from skin biopsies from
patients with sarcoidosis [12]; five of the six cultured iso-
lates were found to be M. avium subsp. paratuberculosis by
PCR [9]. Borrelia burgdorferi was proposed as a possible
agent of sarcoidosis by a Chinese group [16] on the basis of
the elevated titers of serum antibodies in sarcoid patients,
but the same group later concluded that the elevation is a
nonspecific response [42]. Human herpesvirus 8 was pro-
based by an English group [5] to be a cause of sarcoidosis on
the basis of the results of nested PCR, but later research on
these lines by other groups [6, 23, 25] gave negative results.

In Japan, P. acnes was isolated in culture from biopsy
samples of 31 (78%) of 40 lymph nodes from 40 patients
with sarcoidosis. However, there is a problem in evaluation
of the possible connection of this bacterium to the etiology
of sarcoidosis: P. acnes is indigenous to the skin and was
cultured from 21% of 180 tissue samples from patients with
diseases other than sarcoidosis [1]. Contamination by P.
acnes from the skin during biopsy has been suspected, but
a recent study by quantitative PCR [20] concluded that P.
acnes DNA is present, and not as a contaminant, in some
samples from patients without sarcoidosis, but in much
smaller amounts than in patients with sarcoidosis. P. acnes
genomes were abundant in sarcoid lesions only.

III. Quantitative PCR for Detection of
Suspected Bacterial Agents

An international study has been organized by collabo-
rating groups to search for an etiologic connection between
sarcoidosis and certain suspected bacteria [8]. Formalin-
fixed and paraffin-embedded sections of biopsy samples
of lymph nodes, one from each of 108 patients with sarcoidosis
and 65 patients with tuberculosis, together with 86 control
samples, were collected from two institutes in Japan and
three institutes in Italy, Germany, and England. The num-
bers of genomes of P. acnes, P. granulosum, M. tuberculo-
sis, M. avium subsp. paratuberculosis, and Escherichia coli
(as the control) were estimated by quantitative real-time
PCR (QPCR). This collaboration compared the frequencies
of detection and amounts of mycobacterial and propioni-
bacterial DNA in samples from the different institutes and
evaluated the possible etiological link between sarcoidosis
and these bacteria.

Either P. acnes or P. granulosum was found in all but
two of the sarcoid samples. M. avium subsp. paratuberculo-
sis was found in no sarcoid sample. In the different insti-
tutes, M. tuberculosis was found in 0 to 9% of the sarcoid
samples, but in 65 to 100% of the tuberculosis samples. In
sarcoid lymph nodes, the total numbers of genomes of P.
acnes or P. granulosum were far more than those of M.
tuberculosis (Fig. 1). P. acnes or P. granulosum was found
in 0 to 60% of the tuberculosis and control samples, but
the total numbers of genomes of P. acnes or P. granulosum
in such samples were less than those in sarcoid samples.
These results suggest that propionibacteria had resided or
proliferated ectopically in the sarcoid lesions, whether there
was a connection with the disease or not. Propionibacteria
are more likely than mycobacteria to be involved in the
etiology of sarcoidosis, not only in Japanese but also in
European patients with sarcoidosis.

IV. In situ Detection of Propionibacterial DNA

ISH can be used to detect a pathogen within a tissue,
with tissue morphology preserved. Conventional ISH is very
specific, but its sensitivity is limited, and amplification of
the signal often is necessary. Several techniques have been
described for this purpose. Signals can be boosted with
ordinary immunohistochemical amplification methods such
as the use of avidin-biotin complexes or enzyme-labeled
antibodies [15], but the effects may be inadequate. ISH with
PCR uses the principle of PCR by amplifying the target
sequence directly in tissue sections, and the method seems
to be highly sensitive [29]. In signal amplification by cata-
yzed reporter deposition (CARD), biotinylated tyramine is
deposited where the probe has hybridized, amplifying the
hybridization signal [21]. The biotinylated tyramine can
be made visible with avidin labeled with a fluorochrome or
enzyme. The method can detect a single copy of the human
papillomavirus [46], and has been used for identification
in situ of nonviral pathogens such as a bacterium [30] and
fungi [13], the culture of which can be difficult.

Yamada and colleagues [43] have recently detected
P. acnes genomes in histologic sections by ISH that used
CARD for signal amplification with digoxigenin-labeled
oligonucleotide probes that complemented 16S rRNA of
P. acnes. The signals were counted under a microscope, and
the mean counts of granulomatous and nongranulomatous
areas of lymph nodes with sarcoid and tuberculous granu-
ломas were compared with those of control lymph nodes
without granulomas. The results by CARD were further
compared with the results by QPCR in a check of the accu-
racy of the histologic method.

In sarcoid samples, one or several signals were detected
in the cytoplasm of some epithelioid cells in granulomas and
of many mononuclear cells around granulomas (Figs. 2, 3).
The mean signal counts were higher in granulomatous areas
than in other areas of sarcoid lymph nodes (Fig. 4). Even in
nongranulomatous areas of sarcoid lymph nodes, counts
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were higher than in both granulomatous and nongranulomatous areas of tuberculous and control lymph nodes. Correlation (Spearman’s) between the results by QPCR and ISH with CARD was significant (Fig. 5), suggesting that results by ISH with CARD are reliable. Granuloma formation seems to be a critical step in the physiological delayed immune response that stops the spread of noxious and infectious microorganisms [47]. Granulomatous reactions help to contain a number of intra-

cellular pathogens, including bacteria, mycobacteria, fungi, viruses, protozoans, and helminths. In addition, several minerals (beryllium, aluminum, and zirconium) have been suggested as agents that incite hypersensitive granulomas when complexed to proteins of susceptible individuals. The putative agent(s) that causes sarcoidosis must be present at the site of granulomatous inflammation of organs affected by sarcoidosis.

Counts inside sarcoid granulomas being higher than
Fig. 2. CARD with streptavidin conjugated with horseradish peroxidase (HRP) for detection of *P. acnes* in sarcoid (A) and control (B) lymph nodes. Note abundant signals in sarcoid granulomas and the single signal (arrow) in the hyperplastic sinus of the control lymph node.
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Fig. 3. *P. acnes* genomes in and around sarcoid granulomas. (A) CARD with HRP-conjugated streptavidin. (B) Double fluorescence staining of CARD with streptavidin conjugated with fluorescein isothiocyanate and CD68 immunostaining with rhodamine-labeled anti-mouse immunoglobulin.
those outside suggest that \textit{P. acnes} DNA accumulates at the site of granulomatous inflammation, whether the bacterial DNA is from living bacteria or residual bacterial components. The accumulation of \textit{P. acnes} genomes in sarcoid granulomas is evidence more direct than that of earlier studies with bacterial culture and QPCR that this indigenous bacterium is related to the cause of granulomatous inflammation in sarcoidosis.

V. Host Factors

Host factors may be more critical than agent factors in the etiology of sarcoidosis, as already suggested from the phenomenon of the Kveim test, in which a suspension of sarcoid tissues injected intracutaneously causes sarcoid granulomas in patients with sarcoidosis but not in healthy people or patients with other diseases [37].

The inflammatory response in sarcoidosis involves many activated T cells and macrophages [17], with a pattern of cytokine production in the lungs consistent with a helper T-cell type 1 (Th1) immune response triggered by a still undefined antigen(s) [27]. If a propionibacterium caused a particular case of sarcoidosis, it is likely that an antigen arising from the bacterium gave rise to a Th1 immune response in the subject. Ebe and colleagues [7] searched for propionibacterial antigens that evoked cellular immune responses only in patients with sarcoidosis. For this purpose, a λgt11 genomic DNA library of \textit{P. acnes} was screened with sera from patients with sarcoidosis, because such an immune response is usually accompanied by high levels of serum antibodies against the antigen. Of 180,000 plaques screened, two clones coded for an identical recombinant protein, RP35, recognized by sera. RP35, a recombinant protein of 256 amino acid residues with the calculated molecular mass of 28,133 Da, is a fragment (the C-terminal region) of \textit{P. acnes} trigger factor, which has 529 amino acid residues and the calculated molecular mass of 57,614 Da (Fig. 6) [7]. The C-terminal sequence (Asp-463 to Lys-529) seems to be unique to \textit{P. acnes}, with no similarity to sequences of other bacterial proteins deposited in the Swiss-Prot database. The region of Ser-491 to Lys-529 at the C terminus has been found in conformational analysis to be highly antigenic.

RP35 caused sarcoidosis-specific proliferation of peripheral blood mononuclear cells from 9 (18%) of 50 patients with sarcoidosis (Fig. 7) [7]. The same study established that serum levels of IgG and IgA antibodies to RP35 are high for patients with sarcoidosis and other lung diseases. In bronchoalveolar lavage, levels of IgG and IgA antibodies were high in 7 (18%) and 15 (39%), respectively, of 38 patients with sarcoidosis, and in 2 (3%) and 2 (3%), respectively, of 63 patients with other lung diseases. The results of the study suggested that this antigen from \textit{P. acnes} may be responsible for the formation or maintenance of granulomas in patients with sarcoidosis.

In experimental animals, granulomatous lesions can be induced by \textit{P. acnes}. A single intravenous injection of \textit{P. acnes} into mice causes many granulomas in the liver [36, 40, 45], but no granulomas appear in the lungs after such treatment. Pulmonary granulomas can be induced by an intravenous injection of \textit{P. acnes} into sensitized rats [44] and rabbits [19]. In these two studies of experimental pulmonary granulomas, heat-killed \textit{P. acnes} was used as a
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Fig. 6. Alignment of the complete amino acid sequence of the trigger factor of *P. acnes* (A) with the complete sequence of the trigger factor of *M. tuberculosis* (B) from the Swiss-Prot database. Identical amino acids are joined by vertical lines. + indicates a conservative change. Arrows mark the 5'– and 3'– terminal regions of RP35.

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Fig. 7. Response by peripheral blood mononuclear cells (PBMC) to RP35. Fresh peripheral blood was collected from patients with sarcoidosis (Sar; n=50), TB (n=21), and RA (n=32) and from healthy controls (Cont; n=32). The dotted line shows the mean+3SD of the 32 samples from the controls. The horizontal bars show, from bottom to top, the 25th percentile, median, and 75th percentile. The response to RP35 was greater in PBMC from patients with sarcoidosis than PBMC from patients with tuberculosis (p=0.024) or arthritis (p=0.008) and than PBMC from the controls (p=0.001).
Fig. 8. Pulmonary granulomas caused experimentally in mice by a recombinant trigger-factor protein, RP35, of *P. acnes*. Female C57BL/6 mice were sensitized by weekly subcutaneous injections of 50 µg of RP35 emulsified with complete Freund’s adjuvant and examined 3 days after the last sensitization, done 3 weeks after the first sensitization. Note the many granulomas scattered throughout the lung (A, ×22) and a granuloma with many lymphocytes around a core of epithelioid cells with rich eosinophilic cytoplasm (B, ×600).
sensitizer, and challenge by a single intravenous injection of the bacterium was essential for granulomas to form in the lungs. What antigen(s) from \textit{P. acnes} is involved is not known.

RP35 caused pulmonary granulomas in some (25–57\%) of mice sensitized with the protein and complete Freund’s adjuvant (CFA) [26]. An intravenous injection of \textit{P. acnes} as a challenge was not essential for granulomas to form in the lungs. Granulomas were scattered throughout the lungs, especially in subpleural areas (Fig. 8A). The granulomas were composed of a core of epithelioid cells intermingled with a few and surrounded by many mononuclear cells (Fig. 8B). The mechanism of granuloma formation when sensitization is with RP35 only is not known, but this experimental protocol may give a satisfactory model of sarcoidosis in the following ways. First, hypersensitivity to \textit{P. acnes} trigger factor such as has been experimentally induced has already been found in some patients with sarcoidosis. Second, situations resembling an intravenous challenge with \textit{P. acnes} are rare in humans, and sarcoidosis can start in asymptomatic persons without evidence of septicemia.

Experimental models of allergic diseases such as encephalomyelitis [38], thyroiditis [11], and orchitis [39] have been produced by the immunization of animals with self antigens (myelin basic protein, thyroglobulin, and testicular homogenate, respectively) emulsified in CFA, which is essential for the experiment. Autoimmune inflammatory lesions are induced in this way only in the organs from which the self antigens used for the sensitization originated. In the animal model of sarcoidosis, sensitization of mice with RP35 in CFA induces granulomatous inflammation confined to the lungs. This finding suggests that such antigens from \textit{P. acnes} exist in the lungs of mice even before the experiment. \textit{P. acnes} antigens may have cross-reacted with self antigens of the mouse lung, but this is unlikely because more than half of the mice undergoing the same immunization were free from such inflammation, in spite of no differences being found in cellular and humoral immunity against RP35 between the mice with and without pulmonary lesions. Another possibility is that this bacterium normally resides in mouse lungs. Bacterial culture of the lungs suggests this to be more likely. \textit{P. acnes} was cultured from the lungs, liver, and lymph nodes from some untreated normal mice, and culture was most often successful with the lungs. Culture was not done with the mice used in the experiments to avoid possible contamination during sampling. However, there was unexpected concordance in the rate of culture from normal lungs and the frequency of detection of pulmonary granulomas in mice sensitized with RP35. The concordance suggests that mice without granulomas may have been free from \textit{P. acnes} in the normal indigenous flora of their lungs before and during the experiment. Eradication of \textit{P. acnes} by antibiotics might protect mice from granuloma formation caused by this experimental procedure.

The indigenous microflora of human beings and other animals is a remarkably stable ecosystem. The composition of the flora and the numbers of different organisms are various, but for a given host, the flora remains fairly constant with time [4]. A stable microbial system in the respiratory tract acts as a defense mechanism against pathogenic microorganisms. In any organism, there are many factors affecting the establishment of indigenous flora.

The trigger factor is an abundant protein of about 50 kDa found in all eubacteria. Little is known about it. The factor can cross-link to a variety of nascent secretory and cytoplasmic proteins [14] and has ATP-independent chaperone-like activity [35]. Unlike typical molecular
chaperones like some heat-shock proteins, however, the trigger factor does not seem to recognize exposed hydrophobic surfaces [31]. Heat-shock proteins in different bacteria have sequence similarity, and sometimes immunologic cross-reactivity occurs [22]. The trigger factor in different bacteria has little sequence similarity (usually less than 30%), and the sequence most similar to that of P. acnes trigger factor is that of M. tuberculosis [7]. The mechanism by which some patients with sarcoidosis have hypersensitivity to P. acnes trigger factor has not been identified.

VI. A New Concept of Infectious Disease

Once the germ theory of disease was accepted, microbes were considered to be pathogens if they met the stipulations of Koch’s postulate. However, it is apparent that (i) although there are many microbes, most human infections are caused by only a few; (ii) some microbes have been classified as pathogens although they do not cause disease in every host; and (iii) some microbes have been classified as nonpathogens although they did cause disease in certain hosts. In addition, normal individuals harbor, in their mouth and gut, and on their skin, large numbers of microbes that do not cause disease. For that reason, the basic concepts of virulence and pathogenicity of microbes have recently been redefined by Casadevall and Pirofski [3]. They suggested a classification system for pathogens based on the ability to cause damage as a function of the host’s immune response (Fig. 9). To find the cause of sarcoidosis, we need to look at not only pathogenic bacteria such as mycobacteria but also indigenous bacteria of low virulence such as propionibacteria. Koch’s postulates for exogenous infection cannot be applied to a disease caused by endogenous bacteria. Immunologic reactions of Coombs’ type IV hypersensitivity to an antigen of indigenous bacteria classified as a class 6 pathogen may cause granuloma formation.

VII. Summary

Sarcoid granulomas may be formed by a Th1 immune response to one or more antigens of P. acnes indigenous to or proliferating in the affected organ in an individual with a hereditary or acquired abnormality of the immune system (Fig. 10).

VIII. References

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