ABSTRACT
Rheumatoid arthritis is a chronic disease that affects multiple joints. It is considered to be an autoimmune disease in which a T helper (Th)-1 type response has been implicated to play an important pathogenetic role. As osteoclasts, cells that resorb bone, play a crucial part in the bone destruction that occurs in RA, we and others have investigated the pathophysiology of these cells. The findings that interferon (IFN)-γ strongly inhibits osteoclastogenesis and that interleukin (IL)-17 has the ability to enhance osteoclast differentiation have cast doubt on the hypothesis that RA is a Th1 disease. In this review, I describe the relationship between Th cells, the so-called “commander” of the immune response, and RA, mainly from the viewpoint of the environments Th cells create for the excessive differentiation and function of osteoclasts, resulting in the destruction of bone.

KEY WORDS
IL-17, IL-23, osteoclast, receptor activator of NF-κB ligand (RANKL), rheumatoid arthritis (RA), T cell, Th17

INTRODUCTION: WHAT ARE OSTEOCLASTS?
Osteoclasts are specially differentiated giant multinuclear cells of monocyte/macrophage lineage, which decompose bone matrix such as hydroxyapatite and collagen by producing various proteases and acids. These cells have gathered much attention lately because abnormal functions of the cells can lead directly to various clinical problems. In the normal bone, a proper balance is maintained between bone formation by osteoblasts and bone resorption by osteoclasts. If osteoclasts overfunction, however, bone destruction, as is observed in rheumatoid arthritis (RA) and osteoporosis, may occur. On the other hand, reduced activity of osteoclasts causes a hereditary disease called osteopetrosis (Fig. 1a).\(^1\) Bisphosphonates, now used in clinical fields as therapeutic agents for osteoporosis, inhibit both osteoclast differentiation and function, and hence ameliorate osteoporosis.\(^2\)

As is explained in more detail later, several sets of experimental data have shown that “there is no bone destruction of RA without osteoclasts.” Thus, in order to have insight into the pathogenesis of RA, we have tried to understand the conditions in which excessive osteoclastogenesis occurs.

Recently, understanding on osteoclasts has grown rapidly. The identification of the osteoclast differentiation factor has served as the main driving force in this advance. Suda and colleagues revealed that osteoclasts can be differentiated \textit{in vitro} by co-culturing osteoclast precursor cells (monocyte/macrophage lineage cells) with osteoblasts or stromal cells derived from the bone marrow in the presence of vitamin D,\(^3,4\) indicating that osteoblasts produce certain osteoclast differentiation factor(s). About 10 years later, the factor was identified by two independent groups as osteoclast differentiation factor (ODF), or osteoprotegerin ligand (OPGL), respectively.\(^5,6\) Using this cytokine and another cytokine, macrophage colony-stimulating factor (M-CSF), a survival factor for macrophage/macrophage lineage cells, it is now possible to differentiate osteoclasts \textit{in vitro} without the help of osteoblasts. Thus, biochemical analysis with pure osteoclasts was made possible, leading to the recent rapid progress in this field (Fig. 1b).

Interestingly, this TNF family cytokine ODF/
OPGL was found to be identical to the cytokine called receptor activator of NF-κB ligand (RANKL), or TNF-related activation-induced cytokine (TRANCE), which had been reported to be expressed on T lymphocytes the previous year. RANKL has been demonstrated to be expressed on activated T cells and could thus contribute to the activation of dendritic cells that express its receptor (RANK). Thus, activated T cells have been implicated to also stimulate osteoclast precursor cells and promote osteoclastogenesis.

**RHEUMATOID ARTHRITIS AND OSTEOCLASTS**

This finding is particularly important for understanding the pathogenesis of RA. RA is a disease in which multiple joints are damaged by inflammation. Bone and cartilage destruction in the course of persistent inflammation is a serious clinical problem. It is considered to be one of autoimmune diseases, and the activation of T cells recognizing autoantigens is implicated in the pathogenesis. T helper (Th) cells that are "conductors" of the immune response have been divided into 2 subsets (Th1 and Th2) (Fig. 2). The former is the subset responsible for the deprivation of intracellular pathogens such as viruses from the body, and the latter is essential for the protection of the body from extracellular pathogens such as nematodes. RA for some time now has been believed to be a disease in which the Th1/Th2 balance is skewed to Th1. Interferon (IFN)-γ and interleukin (IL)-2, the key cytokines produced from Th1 cells, however, were reported to be barely detected in the affected joints of RA patients. Moreover, IFN-γ receptor-deficient mice exhibited more severe bone destruction in a mouse collagen-induced arthritis (CIA) model that is an animal model of RA, suggesting...
that RA is not in fact a Th1 disease.

The following experimental data indicate that osteoclasts are required in the bone destruction observed in arthritis models. (1) Inflammation was observed in RANKL deficient mice upon transferring serum containing anti-glucose-6-phosphate isomerase antibody (an arthritis model of serum transfer\cite{12}), however, significantly less bone destruction was observed compared to that in wild type mice\cite{13}. (2) The bone destruction in adjuvant-induced arthritis was inhibited by treating the study rats with the inhibitor of RANKL, osteoprotegerin (OPG).\cite{14} These findings led to the conclusion that “there is no bone destruction without osteoclasts.” In the latter report, Kong et al. showed that the RANKL on activated T cells directly acts on osteoclast precursor cells and induces osteoclast differentiation. By the same token, Horwood and his colleagues reported that activated T cells could induce osteoclast differentiation \textit{in vitro}.\cite{15} However, we have to take into consideration the facts that the T cells used by Kong et al. were fixed with formalin, and therefore the effects of humoral factors might be neglected, and that the T cells used by the latter group were derived from humans while the osteoclast precursor cells were from mice, making their system a cross-species co-culture system.

\textbf{IFN-\textgamma\textbf{ STRONGLY INHIBITS OSTEOCLAST DIFFERENTIATION-CONTRADICTION TO THE TH1 HYPOTHESIS OF RA}}

Takayanagi and colleagues reported that IFN-\gamma, which is a representative cytokine of Th1 response, strongly suppressed osteoclast differentiation,\cite{16} a finding contradicting the theory of Kong’s group in case that RA is really a Th1-type disease.

In order to settle the issue, we decided to examine if there are any Th subsets that actually promote osteoclast differentiation. As described above, osteoclasts can be obtained \textit{in vitro} by culturing mice osteoclast precursor cells along with osteoblasts (the co-culture system), and also by culturing the precursor cells alone, first in the presence of M-CSF and then with M-CSF and RANKL (the RANKL/M-CSF system). When we added Th1 or Th2 cells to those culture systems, both Th1/2 cells strongly inhibited osteoclastogenesis.\cite{17} CD25\textsuperscript{+}CD4\textsuperscript{+} T cells, which have received much attention lately as regulatory T cells, did not suppress osteoclast differentiation, but did not enhance osteoclastogenesis either. Thus, the Th subsets that were known at the time could not promote osteoclast differentiation.

\textbf{IL-17-PRODUCING TH CELLS ENHANCE OSTEOCLAST DIFFERENTIATION-THE DISCOVERY OF TH17 SUBSET IN THE CONTEXT OF “OSTEOIMMUNOLOGY”}

We then took notice of a report in which Th cells stimulated by IL-23 produce IL-17.\cite{18} One reason is that Kotake and his colleagues reported that IL-17 was detected in the synovial fluid from RA patients and that it enhanced osteoclastogenesis \textit{in vitro}.\cite{19} Another reason is that IL-23 is a heterodimer cytokine that shares p40 subset with IL-12, which is essential to Th1 differentiation.\cite{20} We hypothesized that

\begin{figure}[h]
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\includegraphics[width=\textwidth]{fig2.png}
\caption{Schematic diagram of Th1 and Th2 differentiation. Naive T helper cells (Thps) stimulated by antigen-presenting cells (APCs) differentiate into either Th1 or Th2 cells, depending on the cytokine profile of the environments. Each subset releases specific cytokines, which inhibit the differentiation of the other subset. As for the provider of IL-4 that is essential to Th2 differentiation, several cells are assumed to play the role, including the Th2 cells themselves.}
\end{figure}
Il17a indicated the significance of Th17 in the pathogenesis of severe bone destruction. In 2005, a new Th subset called Th17 was discovered by using IL-23 instead of IL-12, which might be able to differentiate Th cells that produce IL-17 but not IFN-γ or IL-4. We stimulated Th cells in the presence of IL-23, anti-IFN-γ Ab and anti-IL-4 Ab and obtained the specific cells that produced a large amount of IL-17 but little IFN-γ or IL-4, as we had expected. When we added the cells to the co-culture system of osteoclast differentiation, we were able to enhance osteoclastogenesis for the first time. In 2005, a new Th subset was reported by two independent groups, which is now widely known as Th17, and the method of differentiating the cells was almost the same as that used by us. We not only performed in vitro experiments but also in vivo experiments, and showed that the bone destruction induced by local administration of lipopolysaccharide (LPS) was greatly reduced in both IL-17 deficient mice and IL-23 deficient mice compared to WT mice (Fig. 3). We also showed that in the synovium from RA patients, the expression of RANKL and that of IL-23 (p19 subset and p40 subset) correlated positively, but that those of RANKL and IL-12 (p35 subset) did not (Fig. 4). Thus, we had sought Th subsets that can enhance osteoclastogenesis and encountered the new Th subset, Th17. This was indeed an exciting development and our report first indicated the significance of Th17 in the pathogenesis of the bone destruction in RA.

In 2006, it was reported that IL-6 and TGF-β contributed more to mouse Th17 differentiation than IL-23, and that IL-23 was a growth factor rather than a differentiation factor; however, there is still controversy (See the “Future tasks” section).

One of the mechanisms by which Th17 stimulates osteoclastogenesis is through RANKL induction of osteoblasts via IL-17 signaling. As for the contribution of the RANKL on T cells, Th17 cells did express more RANKL on their surface than Th1 cells but they could not differentiate osteoclasts in the absence of exogenously-added soluble RANKL. Thus, we believe that, at least in our culture system, the RANKL on T cells alone is not sufficient for osteoclastogenesis (Fig. 5). In order to truly examine the hypothesis of Kong et al. described above, we will have to wait for the generation of T-cell specific RANKL deficient mice.

**FUTURE TASKS**

We mainly used cells derived from mice for our experiments and so did the early reports of Th17 cells. Similar IL-17 producing cells have been also discovered in human, but their differentiation factors are reported to be IL-1 and IL-6, instead of IL-6 and TGF-β. In fact, TGF-β had the opposite effect of suppressing human Th17 cell differentiation, indicating a clear species difference. It is always necessary to take these species differences into consideration, and also carefully examine the difference between Th17 cells differentiated in vitro and IL-17 producing Th cells in vivo.

One of the characteristics of Th1/Th2 subsets is their exclusivity; Th1 cells inhibit Th2 differentiation by secreting cytokines and vice versa. This tendency is also observed among Th1/2/17 cells. As this has the structural form of a positive feedback mechanism, microscopically, one of the Th subsets is likely to achieve dominance under specific conditions. However, it is unlikely that all of the cells infiltrating into synovium of an RA patient are Th17 cells, because IFN-γ can be detected in the synovial fluid of these patients, albeit at a very low level. Considering the fact that Th1- and Th2-type cytokines inhibit osteoclast differentiation even at a very low level, does the distribution of Th1/2/17 cells in inflammatory synovium constitute a mosaic pattern? This is an intriguing question in terms of immunopathology. Also, there may be a distinct chronological sequence of Th1/2/17 development in the course of RA development. Concerning this, the study of Jacob and his colleagues is highly thought-provoking; they showed that adjuvant arthritis was worsened when IFN-γ was given 24 hours before administration of the adjuvant, but that the arthritis was ameliorated when the same cytokine was given 24 to 48 hours after immunization with adjuvant, suggesting that IFN-γ may be both
Th17 Cells and Rheumatoid Arthritis

Fig. 4  RANKL expression correlates with that of IL-23 but not of IL-12 in the synovium of RA patients. The relative mRNA expression of RANKL, IL-23A, IL-12A, and IL-12B were standardized using the expression of GAPDH. Note that the RANKL expression positively correlates with that of IL-23A and IL-12B (that constitute the IL-23 heterodimer together) but not with that of IL-12A (that constitutes the IL-12 heterodimer with IL-12B).

Fig. 5  Model of Th17-mediated bone destruction in rheumatoid arthritis. Th17 cells release IL-17, which acts on osteoclastogenesis-supporting cells such as synovial fibroblasts and make them express RANKL. On the contrary, both Th1 and Th2 cells inhibit osteoclast differentiation by releasing IFN-γ and IL-4, respectively. In our experimental system, RANKL expressed on Th17 cells alone is not sufficient for the induction of osteoclast differentiation. In mice, IL-23 is now thought to be a growth factor rather than a differentiation factor and IL-6 and TGF-β are considered to be differentiation factors. In humans, however, IL-1, instead of TGF-β, is implicated in the differentiation of Th17 cells.

beneficial and harmful to RA patients depending on the timing of its administration.30

Since a great deal of attention is now focused on the relationship between this new Th subset Th17 and RA, it is expected that data of Th1/2/17 profile using clinical samples from RA patients will be in hand in the near future. And these results will, without doubt, contribute much to our understanding of RA and the development of better treatment strategies.

REFERENCES


