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GUEST COMMENTARY

Interleukin 4 in Systemic Sclerosis: Not Just an Increase

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The finding of alternative splice variants of cytokines may challenge the existing paradigm of polar type 1 and type 2 cytokine patterns and their distinct roles in disease processes. Generally, type 1 cytokines (interleukin 12 [IL-12], gamma interferon [IFN-γ], and IL-2) drive cellular immunity, whereas type 2 cytokines (IL-4, IL-5, and IL-13) regulate antibody production. In many cell types, type 1 and type 2 cytokines have opposite effects on cellular functions. Recently, alternative splice variants of several interleukins, including IL-4 (1, 3, 29), IL-2 (31), and IL-6 (14), have been identified, and they are being characterized (2–5, 14, 31). The paper by Sakkas et al. (25a) in this issue of *Clinical and Diagnostic Laboratory Immunology* addresses expression of mRNA for a splice variant of IL-4 called IL-4δ2.

IL-4 is a prototypic type 2 cytokine that is made by activated $CD4^+$ and $CD8^+$ T cells and mast cells. It regulates a variety of activities in B cells, T cells, monocytes, and dendritic cells, including immunoglobulin production and isotype switching, and T-cell growth (reviewed in reference 6). In fibroblasts, IL-4 stimulates proliferation (18), chemotaxis (24), extracellular matrix production (9–11, 15, 16, 23), and adhesion molecule expression (8, 22). It also regulates production of other cytokines, such as IL-6, by fibroblasts (8, 9, 25). In contrast, IFN-γ, a prototypic type 1 cytokine, inhibits both proliferation and extracellular matrix production in fibroblasts (21, 27, 28). Thus, it seems reassuring that the fibrotic autoimmune disease systemic sclerosis (Sscl) is associated with an increase in IL-4 but not IFN-γ. Indeed, increased levels of IL-4 are found in the blood (13, 20), bronchoalveolar lavage cells (5), and skin (26) of Sscl patients.

Sakkas et al. (25a) confirm increased levels of IL-4 in the blood of Sscl patients. The authors detected no difference in IFN-γ levels between patients and controls. Using reverse transcriptase-PCR, they found two types of mRNA for IL-4 in peripheral blood mononuclear cells (PBMC) of Sscl patients and controls. The sizes of the two products corresponded to transcripts for full-length IL-4 and for an alternative splice variant of IL-4 (IL-4δ2) described before (1, 3, 29). Cloning and sequencing confirmed the identity of both transcripts. The authors then compared the levels of each mRNA variant for patients and controls. The major novel finding of the present study is that an increase in IL-4δ2 transcript levels is responsible for the higher levels of total IL-4 mRNA in Sscl patients. Interestingly enough, there was no correlation between the level of either transcript and the plasma IL-4 protein level, but there was no correlation between the combined level of both transcripts and the IL-4 protein level. This suggests that both transcripts are translated and secreted; protein detection was by enzyme-linked immunosorbent assay.

The paper of Sakkas et al. comes at a time of interest in expression of IL-4δ2 mRNA in normal and disease states. Expression of IL-4δ2 mRNA is seen in PBMC from all humans tested so far (in our lab, about 50 individuals). It is also found in lung, gut, and thymus tissue (reference 3 and our unpublished data). Both IL-4 and IL-4δ2 are present in placental villi and in amniochorionic and decidual tissue in normal human pregnancy (7). An increase in IL-4δ2 mRNA relative to IL-4 mRNA in PBMC has already been reported for another autoimmune disease, juvenile rheumatoid arthritis (17). A similar increase in IL-4δ2 mRNA has been described for endobronchial biopsies in asthma (12), but the opposite has been observed in bronchoalveolar lavage cells in asthma (4). Also, it has been shown (4, 5) that IL-4δ2 mRNA is increased in bronchoalveolar lavage cells from Sscl patients, along with an overall increase of total IL-4 mRNA relative to IFN-γ mRNA.

Functionally, recombinant IL-4δ2 protein is an IL-4 antagonist in human T cells, B cells, and monocytes, where it probably acts as a competitive inhibitor by binding to IL-4 receptors (2, 3). At the same time, our data indicate that IL-4δ2 is an IL-4 agonist in its effect on fibroblasts, with both IL-4 and IL-4δ2 stimulating fibroblast proliferation and collagen production (reference 5 and our unpublished data). Thus, the question of whether IL-4δ2 should be considered a type 1 or type 2 cytokine arises. It appears that the answer may depend upon the cell type or IL-4 receptors targeted.

Despite this active interest in IL-4δ2 and its potential roles in disease pathogenesis, the corresponding naturally made protein has not been identified. It is difficult to separate IL-4 and IL-4δ2 proteins by conventional methods because of an only 16-amino-acid difference in size, relatively low levels of IL-4 production by mammalian cells, multiple glycosylation forms of IL-4 (30), and very alkaline isoelectric points. Nonetheless, indirect evidence suggests that IL-4δ2 is expressed as a protein. As mentioned above, Sakkas et al. found a correlation between total IL-4 protein and both IL-4 and IL-4δ2 transcripts combined but neither transcript separately. In our own studies, we observed both transcripts bound to polyribosomes by sucrose density gradient centrifugation of total cytoplasmic RNA, whereas neither transcript was found in lighter fractions of unbound RNA.

The functions of IL-4δ2 in vivo have not been characterized. Studies done in vitro with recombinant proteins suggest that alternative splice variants may be antagonists of the corresponding full-length proteins IL-4 (2, 3), IL-2 (31), and IL-6 (14). At the same time, IL-4δ2 is an IL-4 agonist in stimulation of collagen production by fibroblasts (5). It is possible, however, that the splice variants may have functions in vivo in

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addition to antagonism or agonism of the full-length parent cytokines.

Discovery of IL-4δ2 and these initial characterizations of its functional activities make the entire IL-4–IL-4 receptor system even more complicated, particularly as it relates to the concept of IL-4 as a type 2 cytokine. The effect of IL-4 may depend on the ratio of the full-length IL-4 to IL-4δ2, the cell type targeted, or the IL-4 receptors bound (19). If IL-4 predominates, then a typical response to type 2 cytokines should be expected. If IL-4δ2 predominates, then the effect might mimic that of type 1 cytokines (anti-IL-4) on hematopoietic cells yet mimic responses to type 2 cytokines in certain nonhematopoietic cells.

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