Introduction

Sarcoidosis is defined as “a multisystem disease of unknown cause characterized by the formation in all or several affected tissues of epithelioid-cell tubercles without caseation” (1). The lungs are the organ most often affected and symptoms of pulmonary sarcoidosis include cough, dyspnea, wheezing and chest pain (2). Diagnostic criteria for sarcoidosis include radiological features, such as bilateral hilar lymphadenopathy and pulmonary infiltration, presence of non-caseating granulomatous inflammation and exclusion of similar diseases (3,4). A widely used biomarker for disease activity in sarcoidosis is...
the soluble IL-2 receptor (sIL-2R). The full human membrane-associated IL-2 receptor consists of three subunits (α, β and γ) and is expressed on activated T cells, while an intermediate affinity form containing only the β and γ chains is found on resting T cells (5). Under certain conditions the α-chain is released from the membrane as sIL-2R. The serum level of this molecule is used to assess disease severity in a number of inflammatory conditions, including sarcoidosis. While the value of sIL-2R as a biomarker is reasonably well established, little is known about its biological role in sarcoidosis. After a brief discussion of sIL-2R as a biomarker, this paper will review the molecular and cellular origin of sIL-2R, its functional effects and potential mechanisms of action, and finally, its potential role in sarcoidosis.

sIL-2R as a Biomarker for sarcoidosis severity

The serum level of sIL-2R is assessed using a quantitative ELISA and has been found to correlate with various disease aspects. Several investigations have shown that the serum level of sIL-2R is significantly higher in sarcoidosis patients than in healthy controls (6-9). Additionally, sIL-2R levels are higher in patients with active disease compared to inactive disease (7, 8, 10-13). In contrast, the level of sIL-2R does not clearly differentiate between patients with different radiological stages (12) and, additionally, data reported on sIL-2R in relation to disease manifestation conflict (14,15). Furthermore, serum sIL-2R levels are significantly lower in treated than in untreated patients (10, 11, 16) and significantly decrease after treatment (6, 17, 18). Interestingly, while some reports indicate a correlation between the serum levels of sIL-2R and ACE, another biomarker for sarcoidosis, (10, 12, 19), others could not reproduce these findings (6, 14, 20). Associations between sIL-2R and other sarcoidosis biomarkers, such as soluble CD27 (21), C-reactive protein (12), α1-acid glycoprotein (12), chitotriosidase (9) and soluble CD163 (22), have been reported, but these findings still need to be reproduced. The serum sIL-2R level has also been associated with certain lung function parameters including vital capacity and carbon monoxide diffusion (8), and, improvements in lung function as a result of methotrexate therapy were found to correlate with decreases in sIL-2R levels (18).

Finally, a relation has been reported between the serum sIL-2R level and outcome of 67Gallium scans (6, 23) and, while sIL-2R levels were significantly higher in patients with positive 18F-FDG PET scan results (24, 25), they did not directly correlate with standardized uptake values (17, 19). Thus, while above data indicate that sIL-2R can be a valuable biomarker for diagnosis and therapy of sarcoidosis, the exact role of sIL-2R in the immunopathology of sarcoidosis has not been elucidated.

Cellular origin of sIL-2R

While various studies have reported that T cells produce sIL-2R (26-33), they only produce notable amounts of sIL-2R after activation (26, 31, 32), with the exception of regulatory T cells which do not require (in vitro) activation (31, 32). Other cells of the immune system, including dendritic cells (34), macrophages and monocytes (29, 35) and B cells (26) can produce sIL-2R as well. While T cells are considered the main source of sIL-2R in sarcoidosis (13, 18, 21), there is some evidence indicating that macrophages also contribute to sIL-2R production in sarcoidosis (7, 8).

Generation of sIL-2R

The soluble form of the IL-2 receptor is most likely produced through enzymatic cleavage of membrane IL-2Rα. Experimental evidence argues against other mechanisms, such as cell death (36), separate genes for membrane IL-2R and sIL-2R (37) or differential mRNA splicing (37). Furthermore, it has been reported that membrane IL-2Rα levels decrease as sIL-2R levels increase, which is consistent with enzymatic cleavage (38, 39). In contrast, continuous synthesis and degradation of membrane IL-2Rα has been reported and, consequently, not all membrane IL-2Rα is released as sIL-2R (28). Additionally, using an enzymology approach, it was estimated that sIL-2R terminates between residues 187 and 192 of the membrane IL-2Rα molecule (40). Several cellular structures potentially involved in sIL-2R production, including lysosomal enzymes, proteases and serum components, were studied, but none of these processes were found to be essential
for sIL-2R production (40). Furthermore, based on analysis of enzyme kinetics, it has been suggested that IL-2Rα release as sIL-2R is a first-order, non-saturable process, which could be consistent with both enzymatic proteolysis and autocatalysis (41). Several enzymes responsible for cleavage of membrane IL-2R have been proposed: Der p 1 produced by *Dermatophagoides pteronyssinus* (42), neutrophil elastase (43) and metalloproteinases 2 and 9 (30, 44). Regardless of all data above, it has to be concluded that despite over 30 years of research, the exact molecular mechanism leading to generation of sIL-2R remains to be determined.

**Functional effects of sIL-2R**

Theoretically, sIL-2R could have three functional effects on the immune response. It could inhibit the immune response, stimulate the immune response or have no effect. The majority of studies (30, 32-34, 45-47) reported inhibitory effects, such as reduced T cell proliferation, decreased cytotoxicity and increased apoptosis. On the other hand, some studies observed immunostimulatory effects, including increased peripheral blood monocyte proliferation (48), STAT5 phosphorylation (39) and Th17 cell numbers (49). Furthermore, two studies (31, 50) observed no effect of sIL-2R on the immune response. However, Lindqvist and colleagues (32) pointed out a methodological limitation of these studies, namely that their sIL-2R was derived from an in vivo source, and consequently, might be bound to IL-2. Certain variables, such as the number of cells in culture (46) and the medium constituents and stimulatory signals (30) may modulate sIL-2R’s effect. Since none of the cells in these studies were obtained from sarcoidosis patients, these data should be considered broadly applicable, not specific to sarcoidosis.

**Mechanisms of action**

Since the functional effects of sIL-2R remain subject to controversy, the underlying mechanism of those functional effects likewise is unknown. However, a wide range of theories has been proposed including reduced IL-2 receptor density (51), local confinement of the inflammation (51), interaction with membrane IL-2 receptor (46), prevention of IL-2-mediated activation-induced cell death (48), interference with IL-2 feedback (49, 52) and inhibition of membrane IL-2Rα expression (47). While there is evidence in favor of and against any of these mechanisms, the following discussion is limited to the three most plausible mechanisms, namely IL-2 sequestration, prolongation of IL-2 half-life and induction of a structural change in IL-2 (Figure 1).

Probably the most commonly proposed mechanism of action of sIL-2R is sequestration of IL-2. This was already suggested in the original publication on the discovery of sIL-2R: “the release of soluble IL-2R by activated lymphocytes might serve an immunoregulatory role by competing with cellular IL-2R for the growth factor IL-2 and thus down-regulating the immune response” (36). Support for this theory is found in sIL-2R’s ability to bind IL-2 (38, 53) with an affinity between 10 (40) and 30 nM (54). The major limitation is that this reported affinity of sIL-2R for IL-2 is approximately 1000 times lower than the affinity of the full membrane IL-2 receptor for IL-2 (10^{-11} M, 5). As such, it is unlikely that sIL-2R can effectively compete for binding of IL-2 with the full membrane receptor. However, only activated T cells express the full heterotrimeric IL-2 receptor, while resting T cells express only the IL-2Rβγ. The affinity of this dimeric receptor is only 10 times higher than that of sIL-2R (10^{-9} M, 5). Consequently, while sIL-2R may not be able to affect activated T cells, it might negatively impact activation of resting T cells by limiting IL-2 availability. One fundamental question related to this mechanism is whether or not resting T cells expressing only the intermediate affinity receptor can respond to IL-2. Since IL-2Rα is not associated with intracellular signaling pathways, it could be argued that cells should still be able to respond to IL-2 in its absence. Additionally, since T-cell activation results in IL-2 expression and, in turn, intracellular signaling mediated by the IL-2 receptor results in expression of the heterotrimeric IL-2 receptor. However, if resting T cells do not respond to IL-2 in the same way as active T cells, it is unlikely that competition by sIL-2R for IL-2 would have an effect.

sIL-2R theoretically also has immunostimulatory potential. Binding of sIL-2R to IL-2 does
not have to prevent IL-2 from interacting with the membrane IL-2 receptor, but rather may protect it from degradation by serum proteases. As such, sIL-2R would behave as a carrier protein for IL-2. This theory was originally proposed by Caruso and colleagues (51) and experimental evidence was provided by Kobayashi and associates (55), who conducted both in vivo and in vitro experiments that indicate that sIL-2R prolongs IL-2’s half-life.

Finally, Maier and colleagues (48) suggested that binding of sIL-2R to IL-2 might promote IL-2 signaling, by analogy with the IL-6 and IL-15 systems (56, 57). Indeed, it was found that membrane IL-2Rα induces a conformational change in the IL-2 molecule which increases its affinity for IL-2Rβ (58). Consequently, it is possible that sIL-2R induces the same conformational change and, as such, the affinity of IL-2Rβ would be higher for the sIL-2R-IL-2 complex than for IL-2 alone.

**IL-2 and sIL-2R in sarcoidosis**

While the immunopathology of sarcoidosis is very complex and many aspects are still unclear, it is in essence a Th1-mediated granulomatous inflammatory disorder (1). There is a considerable amount of evidence indicating that IL-2 plays a vital role in sarcoidosis. First of all, through upregulation of the IL-12 receptor β2 subunit IL-2 promotes the development of Th1 cells (59, 60). Secondly, abnormalities in the IL-2 system have been observed in sarcoidosis, including increased IL-2 release by T cells (61) and increased expression of membrane IL-2Rα (62-64).
Thirdly, therapeutic administration of IL-2 results in development of sarcoidosis-like disease in HIV patients (65, 66) and a case study reported worsening of symptoms in a patient with sarcoidosis after IL-2 immunotherapy (67). Finally, a recent study reported dysregulation of IL-2 release in sarcoidosis and, interestingly, that disease resolution was accompanied by restoration of IL-2 release (68). As such, these studies highlight the importance of IL-2 in the immunopathology of sarcoidosis.

Although the exact functional effect of sIL-2R is not yet fully understood, it is most likely that in sarcoidosis sIL-2R interacts with IL-2. Three possible roles for sIL-2R in the immunopathology of sarcoidosis can be envisioned. First of all, sIL-2R could contribute directly to the immunopathology of sarcoidosis. By prolonging the half-life of IL-2 or by increasing its affinity for IL-2Rβ, sIL-2R could stimulate IL-2 signaling, which would promote the inflammatory process and might also result in proliferation of T cells specific for other antigens (Figure 2A). Alternatively, sIL-2R might inhibit T cell proliferation through IL-2 sequestration which would impair clearance of disease-promoting antigens (Figure 2B). On the other hand, the presence of sIL-2R might reflect an ongoing process to limit inflammation, which apparently in sarcoidosis is ineffective. By preventing activation of resting T cells through IL-2 sequestration, sIL-2R would reduce inflammation. However, since the inflammation in sarcoidosis does not resolve despite high sIL-2R levels, it is apparently ineffective. Finally, it is possible that sIL-2R does not exert a major biological effect and is only useful as a biomarker.

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**Fig. 2.** Potential mechanisms explaining how sIL-2R might contribute to the immunopathology of sarcoidosis. A) Binding of sIL-2R to IL-2 increases the affinity of IL-2 for the intermediate affinity receptor. Consequently, the sIL-2R/IL-2 complex can activate resting (potentially autoreactive) T cells, which then proliferate. B) Binding of sIL-2R to IL-2 sequesters IL-2 from resting cells only expressing the intermediate affinity receptor. As a result, T cell proliferation is reduced and antigen clearance is affected. (s)IL-2Rα: red; IL-2Rβ: purple; IL-2Rγ: blue; IL-2: purple sphere
Biological role of sIL-2R in sarcoidosis

While the existing literature provides substantial information on sIL-2R and sarcoidosis, there are several unresolved issues. First of all, efforts should be made to identify the enzyme responsible for the cleavage of membrane IL-2Rα, as this might lead to the development of drugs that could inhibit this enzyme and, consequently, selectively block sIL-2R production. Furthermore, the inconsistencies reported on the immunological effect of sIL-2R impede our understanding of its role in sarcoidosis and a study investigating factors that moderate sIL-2R’s effect is highly desirable. Additionally, while there is some evidence for the various mechanisms of action of sIL-2R, more substantial experimental proof is required. Finally, studies in patients could determine if administration of sIL-2R has a functional role in the immunopathology of sarcoidosis, but due to ethical constraints those studies would not be feasible.

CONCLUSION

Based on its correlation with various disease aspects, including disease activity, response to treatment, lung function, radiological examinations and other biomarkers, sIL-2R is a valuable biomarker for sarcoidosis. Additionally, based on the immunological effect of sIL-2R, it is possible that sIL-2R has a biological role in sarcoidosis. However, several aspects of sIL-2R’s function remain unclear and future research will have to address these. Depending on its role, sIL-2R might become a therapeutic target. If sIL-2R is found to be play a protective role, future therapies might include administration of exogenous sIL-2R. Alternatively, if sIL-2R actively promotes disease development, inhibitors of the enzyme responsible for sIL-2R production could be therapeutically useful. In conclusion, aside from its potential as a biomarker, sIL-2R might play a biological role in sarcoidosis and become a target for future therapies.

REFERENCES


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