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Cytokines in Alopecia Areata: Contrasting Cytokine Profiles in Localized Form and Extensive Form (Alopecia Universalis)

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Recent studies have suggested that cytokines play a critical role in the pathophysiology of alopecia areata; however, no information is available regarding the difference in cytokine profiles in these patients.

Serum levels of cytokines, including interferon γ (IFN-γ), tumor necrosis factor α, interleukin 1α (IL-1α), IL-2, IL-4, and IL-6, were measured using radioimmunoassay or enzyme-linked immunosorbent assay techniques in patients with the localized form and the extensive form (alopecia universalis).

The serum levels of IL-1α and IL-4 were significantly elevated in patients with the localized form. In contrast, the serum levels of IFN-γ and IL-2 were significantly elevated in patients with the extensive form.

These results indicate that immune responses in the localized form and the extensive form of alopecia areata are regulated by Th2 cytokines and Th1 cytokines, respectively. ***Key word: T helper cell.***

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Although the pathogenesis of alopecia areata (AA) is still unknown, evidence is accumulating that AA is an immunologically mediated disease (1–3). The potential importance of cytokines in the pathogenesis of AA has been suggested by recent findings: follicular epithelial cells expressing intercellular adhesion molecule-1 (ICAM-1) and MHC class II molecules such as HLA-DR (4); intense expression of E-selectin, vascular cell adhesion molecule-1, and ICAM-1 on the perifollicular dermal vessels (4); and effectiveness of cyclosporin A in this condition (5). If the patterns of cytokine release are causally related to be phenotype of disease expression, we would expect that a cytokine profile in the localized form may be different from that in the extensive form. Nevertheless, no information is available regarding the difference in cytokine profiles between patients with the localized form and those with the extensive form.

To clarify factors predictive of outcome, serum levels of interferon γ (IFN-γ), tumor necrosis factor α (TNF-α), interleukin 1α (IL-1α), IL-2, IL-4, and IL-6 in patients with the localized form of AA (LAA) were compared with those in alopecia universalis (AU). Contrasting cytokine profiles were observed between the two groups.

MATERIAL AND METHODS

Seven patients with LAA (mean age: 24 years) had a few patches (less than 20% hair loss) and 7 patients with AU (mean age: 21 years) were included. The duration of hair loss in the two groups varied from 0.3 to 3.0 years (mean: 1.2 years) and 1.5 to 15.0 years (mean: 5.9 years), respectively. No patients had used any systemic medications for AA for at least 3 weeks or therapies such as PUVA, which could have a prolonged effect on cytokine levels, for at least 6 months before this study. We excluded patients who had other types of illnesses such as autoimmune diseases that could affect the outcome of the study and those who had received treatment with systemic steroids and other immunosuppressive medications. Seven healthy subjects, ranging from 20 to 22 years of age (mean age: 21 years) served as controls. The serum samples obtained for the assay of these cytokines were stored at −80°C before the assay.

Cytokine assay

The serum levels of IFN-γ, TNF-α, IL-1α, and IL-2 were measured with commercially available radioimmunoassay kits (IFN-γ assay system: Centocor, Malvern, Pa; TNF-α assay system: Medgenix, Fleurus, Belgium, IL-1α assay system: Amersham Corp., Arlington Heights, IL; and IL-2 assay system: Medgenix, Fleurus, Belgium), as described previously (6). The assays for IL-4 and IL-6 were performed with commercially available enzyme-linked immunosorbent assay kits (IL-4 assay system: Genzyme Corp., Boston, MA; and IL-6 assay system: Toray Corp., Tokyo). All assays were performed according to the manufacturer's instructions. The cytokine concentrations of the sample were determined by interpolation from standard curves derived from recombinant human cytokines added to normal human serum. The assays were sensitive to 0.02 U/ml for IFN-γ, 7.5 pg/ml for TNF-α, 5 fmol/ml for IL-1α, 0.5 U/ml for IL-2, 46 pg/ml for IL-4, and 25 pg/ml for IL-6, respectively. Each cytokine assay system was shown not to cross-react with other cytokines. All assays were performed in a blind fashion on coded samples by an investigator who was not informed of the patients' clinical status, after the collection of all samples had been completed.

Statistical analysis

The data are expressed as mean ± standard deviation. Comparisons were performed by the Mann-Whitney U-test. The data were considered statistically significant if p values were less than 0.05.

RESULTS

The results of serum cytokine levels and statistical analysis are shown in Table I.

LAA vs controls

Serum levels of IL-1α (29.8 ± 3.6 vs 13.1 ± 2.8 fmol/ml) and IL-4 (152 ± 118 vs < 46 pg/ml) in patients with LAA were significantly higher than those in controls. No significant differences were observed in serum levels of IFN-γ (0.032 ± 0.027 vs < 0.020 U/ml), TNF-α (8.3 ± 0.9 vs 7.6 ± 0.2 pg/ml), IL-2 (< 0.50 vs 0.53 ± 0.30 U/ml), and IL-6 (62 ± 98 vs < 25 pg/ml) between patients with LAA and controls.

Table I. *Comparisons of serum cytokine levels in patients with the localized form of alopecia areata, the extensive form (alopecia universalis) and controls*

	IFN-γ (U/ml)	TNF-α (pg/ml)	IL-1α (fmol/ml)	IL-2 (U/ml)	Il-4 (pg/ml)	IL-6 (pg/ml)
Localized form						
1	<0.020	8.2	34.0	<0.50	165	<25
2	0.032	<7.5	28.0	<0.50	<46	<25
3	<0.020	9.4	33.5	<0.50	330	>25
4	<0.020	9.6	26.0	<0.50	140	<25
5	<0.020	7.5	25.5	<0.50	290	285
6	<0.020	<7.5	28.5	<0.50	<46	<25
7	<0.020	8.2	33.0	<0.50	50	<25
Mean ± SD	0.032 ± 0.027	8.3 ± 0.9	29.8 ± 3.6	<0.50	152 ± 118	62 ± 98
Extensive form						
1	<0.020	<7.5	13.6	<0.50	<46	<25
2	0.084	<7.5	16.7	0.72	<46	<25
3	0.030	<7.5	13.8	2.05	<46	83
4	0.020	<7.5	20.5	0.73	<46	143
5	0.036	<7.5	14.4	2.45	<46	<25
6	0.020	<7.5	7.9	2.95	94	<25
7	0.034	<7.5	13.7	0.59	<46	<25
Mean ± SD	0.035 ± 0.023	<7.5	14.4 ± 3.8	1.43 ± 1.02	53 ± 18	50 ± 46
Controls						
1	<0.020	8.1	7.1	<0.50	<46	<25
2	<0.020	<7.5	15.0	0.59	<46	<25
3	<0.020	<7.5	13.5	<0.50	<46	<25
4	<0.020	7.5	13.5	<0.50	<46	<25
5	<0.020	<7.5	13.5	<0.50	<46	<25
6	<0.020	7.5	13.5	<0.50	<46	<25
7	<0.020	<7.5	13.5	<0.50	<46	<25
Mean ± SD	<0.020	7.6 ± 0.2	13.1 ± 2.8	0.53 ± 0.3	<46	<25

Statistical analysis was performed by the Mann-Whitney U-test. *$p<0.05$, and **$p<0.01$.

AU vs controls

Serum levels of IFN-γ (0.035 ± 0.023 vs < 0.020 U/ml) and IL-2 (1.43 ± 1.02 vs 0.53 ± 0.30 U/ml) in patients with AU were significantly higher than those in controls. There were no significant differences in levels of TNF-α (<7.5 vs 7.6 ± 0.2 pg/ml), IL-2 (1.43 ± 1.02 vs 0.53 ± 0.30 U/ml), IL-4 (53 ± 18 vs < 46 pg/ml), and IL-6 (50 ± 46 vs < 25 pg/ml) between patients with AU and controls.

LAA vs AU

Serum levels of TNF-α (8.3 ± 0.9 vs < 7.5 pg/ml) and IL-1α (29.8 ± 3.6 vs 14.4 ± 3.8 fmol/ml) in patients with LAA were significantly higher than those in patients with AU, while levels of IL-2 (<0.50 vs 1.43 ± 1.02 U/ml) in patients with LAA were significantly lower than those in patients with AU. There were no significant differences in levels of IFN-γ (0.032 ± 0.027 vs 0.035 ± 0.023 U/ml), IL-4 (152 ± 118 vs 53 ± 18 pg/ml), and IL-6 (62 ± 98 vs 50 ± 46 pg/ml) between patients with LAA and AU.

There was an inverse correlation between disease duration and the serum levels of IL-1α, but not other cytokine levels.

DISCUSSION

In this study we found that there was a difference in a cytokine profile between LAA and AU. This provides evidence to indicate that immune responses in LAA and AU are regulated by a distinct pattern of cytokines, although it remains unclear whether these two forms of AA are totally distinct in their pathogenesis or whether they may simply represent different manifestations of a single disorder. One may argue that our findings may be due to difference in the duration of disease between the two groups. This possibility is, however, unlikely, because there was no significant correlation between the duration of disease and the serum levels of IFN-γ, TNF-α, IL-2, IL-4, and IL-6.

Mouse $CD4^+$ helper T cells are divided into two subsets based on differences in the pattern of cytokine production: type 1 T helper (Th1) cells produce IFN-γ and IL-2 but not IL-4 and IL-5, whereas type 2 T helper (Th2) cells produce IL-4 and IL-5 but not IFN-γ and IL-2 (7). T-cells with a Th1 or Th2 functional pattern have also been identified in humans (8, 9). Our finding that the serum levels of IL-4 were significantly elevated in patients with LAA, whereas the serum levels of IFN-γ and IL-2 were significantly elevated in patients with AU, indicates that the cytokine patterns in patients with LAA are similar to a Th2 cytokine profile, whereas those in patients with AU are similar to a Th1 cytokine profile. These findings can be interpreted as an indication that Th1-type cytokines may be critical for the progression to the extensive form and that Th2-type cytokines may exert a more subtle influence on the inhibition of a cell-mediated attack on hair follicles. In fact, there are several pieces of evidence to indicate the involvement of Th1-type cytokines in inflammatory responses

observed in AA: the presence of IFN-γ is inferred on the perifollicular infiltrates (1) and ICAM-1 and HLA-DR, which are shown to be induced by IFN-γ, are intensely expressed on follicular epithelium (4). More recently, Hoffmann et al. (10) reported that Th1-type cytokine mRNA levels are increased in untreated AA of the totalis type. They hypothesized that increased lesional levels of IL-10 after topical immunotherapy using diphenylcyclopropenone application resulted in an inhibitory effect on lesional T lymphocytes. These findings indicate that Th1-type cytokines play a critical role in the pathogenesis of AA. Taken together, in patients with AA a Th2 cytokine profile may indicate a good prognostic value, while a Th1 cytokine profile appears to indicate a poor prognosis.

Consistent with our results, recent studies have suggested that Th2 cells may serve as self-protection mechanism(s) by counteracting the tissue-damaging effects of Th1 cells: e.g. IL-4 prevents onset of diabetes in nonobese diabetic mice, which spontaneously develop an autoimmune form of diabetes associated with insulitis (11); and cells of the Th2 cytokine phenotype prevent LPS-induced lethality during murine graft-versus-host reaction (12). As we were unable to sequentially examine cytokine profiles in patients with LAA who finally progressed to AU and in those who did not, further studies are certainly needed to strengthen our hypothesis that a Th2-like response could inhibit the tissue-damaging effects of Th1 cells on hair follicles in AA.

Although our results are drawn from a very low patient number and several factors, such as the presence of soluble cytokine receptor or various cytokine-binding proteins, influence the estimates of cytokine levels provided by immunoassay, the measurement of serum cytokines in patients with AA may be useful in discriminating those likely to progress to AU from the remaining LAA, or as a prognostic indicator. In addition, the characterization of the role of key cytokines essential to sustaining inflammation may lead to new approaches to therapeutic intervention.

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