

Serum T helper 1 cytokine levels are greater in patients with alopecia areata regardless of severity or atopy

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Summary

Background. Alopecia areata (AA) is an organ-specific autoimmune disease characterized by folliculotropic T-cell infiltrates around anagen-stage hair follicles. The role of T helper (Th)1 and Th2 cytokines in the pathogenesis of AA have not been established.

Aim. To determine whether serum cytokine profiles define the severity of the AA phenotype or are affected by co-existent atopy.

Methods. In total, 17 serum cytokines were measured and compared in 269 patients with AA of varying severity with and without atopy and 18 unrelated controls.

Results. Of the 269 patients with AA, 96% had active disease and 54% were atopic. The disease phenotype was transient patchy AA in 27 patients, persistent patchy AA in 89 and alopecia totalis or alopecia universalis in 153. Levels of Th1, interleukin (IL)-1 receptor antagonist (ra) and IL-8 levels were higher in all patients with AA than in controls. IL-1 α , IL-12 and tumour necrosis factor- α levels were higher in patients with AA and atopy than in patients with AA without atopy.

Conclusions. Increased Th1 serum cytokines (IL-2, IL-12 and interferon- γ) and IL-1ra levels are associated with AA regardless of disease severity or the presence of atopy.

Introduction

Alopecia areata (AA) is an organ-specific autoimmune disease characterized by T-cell infiltrates and cytokine production around anagen-stage hair follicles.^{1,2} AA affects 1.7% of the population and shows no gender or ethnic predilection.³ The severity of hair loss or disease phenotype is variable and can fluctuate over time; regrowth occurs in most patients. Patchy AA is characterized by well-defined local patches of hair loss. Alopecia totalis (AT; total scalp hair loss) and alopecia universalis (AU; hair loss over the scalp and body), are the most severe phenotypes.

T cells secreting cytokines are grouped by their ability to promote cellular [T helper (Th)1] or humoral (Th2) reactions. Evidence for the role of cytokines and T-cells in AA includes the beneficial effects of systemic steroids and calcineurin inhibitors and the finding of decreased interleukin (IL)-2 and increased IL-4 serum levels after diphenylcyclopropenone treatment.^{4–6} CD8+ and CD4+ cells have been found to contribute to AA in the severe combined immunodeficiency (SCID) mouse scalp-transplant model.⁷ The association of AA with atopy, defined as atopic dermatitis (AD), allergic rhinoconjunctivitis or asthma, which is mediated by Th2 cytokines, has been reported in 10–60% of patients.^{8,9} IL-4 is found in sera from patients with AA and sera from C3H/HeJ AA mice,^{10,11} but is lower than levels found in sera from patients with AD.¹² Th1 cytokines, interferon (IFN)- γ , IL-2, IL-12 and tumour necrosis factor (TNF)- α , have also been found in sera and lesional biopsy samples from patients with AA.^{6,10–14} Katagiri *et al.* reported that IFN- γ was lower in both patients with AA and patients with atopy compared with controls, suggesting

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an association between AA and atopy.¹² Thus, the relative role of Th1 vs. Th2 cytokines in AA is not clear. To determine if Th1 or Th2 cytokines play an important role in AA and whether the presence of atopy influences these cytokine profiles, we measured serum cytokines in patients with AA and stratified the results by phenotypic severity and atopy.

Methods

Patients signed consent forms approved at each institution and the study was conducted in accordance with the principles of the Declaration of Helsinki.

Patients and data collection

Study participants self-registered via the National Alopecia Areata Registry (NAAR; <http://www.AlopeciaAreataRegistry.org>). Patients and unrelated controls recruited by NAAR provided epidemiological data, had a skin examination and contributed sera and DNA.¹⁵

Samples were collected from 269 patients with sporadic AA [197 (73%) women, 72 (27%) men; mean $\pm$ age 39 ± 18.8 years] and 18 unaffected and unrelated control subjects [12 women (67%), 6 men (33%); mean $\pm$ age 44 ± 13.6 years]. Approximately 90% ($n = 242$) of patients with AA and 56% ($n = 10$) of controls were white. The 269 patients with AA were stratified by phenotype and disease severity: 27 had a history of transient AA (AAT) for < 1 years, 89 had a history of persistent AA (AAP) on the scalp for > 1 years and 153 had AT or AU. Controls were recruited from one of four sources: (i) volunteers who were notified through advertisements at various sources such as the National Institutes of Health or the National Alopecia Areata Foundation (NAAF) website; (ii) volunteers obtained from NAAF annual conferences; (iii) members of the research team who were involved in NAAR or NAAF; and (iv) unaffected, unrelated family members or friends of patients who had participated in NAAR. Control subjects were asked to confirm a lack of AA in blood relatives. Because of the small number of controls, we were unable to match them to cases on age, gender or presence of atopy. Patients were also grouped according to number of AA episodes (1, 2–10, or > 10 AA episodes). Active AA status was defined as positive if a patient had active disease at the time of blood collection. Self-reported atopy status was defined as positive if a patient had AD, asthma, allergic rhinoconjunctivitis or any combination of these conditions.

Cytokine analysis

Serum cytokine levels were measured using a multi-analyte proteome array (SearchLight; Pierce Biotechnology, Inc., Rockford, IL, USA), which allowed simultaneous measurement of the following cytokines under identical conditions to an established minimum detectable level: IL-1 α (2 pg/mL), IL-1 β (1 pg/mL), IL-1 receptor antagonist (ra) (7.8 pg/mL), IL-2 (4 pg/mL), IL-4 (3.9 pg/mL), IL-5 (2 pg/mL), IL-6 (2 pg/mL), IL-8 (2 pg/mL), IL-9 (30.5 pg/mL), IL-10 (2 pg/mL), IL-12p70 (2.9 pg/mL), IL-13 (39.1 pg/mL), IL-15 (2 pg/mL), IL-18 (10 pg/mL), IFN- γ (2 pg/mL), TNF- α (11.7 pg/mL) and RANTES (regulated upon activation, normal T-cell expressed and secreted) (2 pg/mL). Duplicate samples were tested to ensure that variation between wells was minimal.

Statistical analysis

We compared serum cytokine levels of all patients with AA and of the three phenotypes (AAT, AAP, AT/AU) with controls. Because serum cytokine levels were skewed and the data contained several outliers, a logarithmic transformation was applied. Because of the nature of these data, two medians were calculated: the overall median and the median of the dispersion of values above the minimum detectable value. The Kruskal–Wallis test was used to compare the median serum cytokines between AA subgroups (AAT, AAP, AT/AU) and controls, and between number of episodes, for both the overall and the dispersion data (Tables 1 and 2, Table S1 online). The P -value from the Kruskal–Wallis test is an indicator of statistically relevant differences between the distributions of serum cytokine levels in the atopy subgroups. Jitter plots were used to illustrate the range of possible cytokine values for various cytokines in AA subgroups vs. those in healthy controls (Fig. 1, shown on a logarithmic scale). Finally, the Wilcoxon rank sum test was used to compare overall median serum cytokine levels between patients with and without atopy (Table S2). $P < 0.05$ was considered significant. All analyses were performed using the software programs SAS (version 9.1 for Windows; SAS Institute, Cary, NC, USA) and S-Plus (version 7; Insightful Corporation, Seattle, WA, USA).

Results

Of the 269 patients with AA in this study, 96% (258 patients) reported having active disease at the time blood samples were drawn and all unrelated controls

Table 1 Comparison of overall median serum cytokine levels in alopecia areata subgroups and controls.

Cytokine	Controls (n = 18)	AA subgroup			P value*
		AAP (n = 89)	AAT (n = 27)	AT/AU (n = 153)	
IFN- γ					
Median, pg/mL	1.54	2.02	2.15	2.05	0.084
Range, pg/mL	0.69–2.71	0.68–4.66	0.69–4.21	0.68–5.49	
Above DV, %†	61.1	83.2	74.1	85.0	
IL-2					
Median, pg/mL	2.01	2.70	2.95	2.70	0.174
Range, pg/mL	1.36–3.91	1.36–5.81	1.36–5.09	1.36–5.00	
Above DV, %	55.6	71.9	66.7	70.6	
IL-12					
Median, pg/mL	1.06	1.29	1.62	1.16	0.106
Range, pg/mL	1.06–2.33	1.06–4.58	1.06–4.08	1.06–4.03	
Above DV, %	33.3	55.1	51.8	51.6	
IL-15					
Median, pg/mL	0.69	0.69	0.69	0.69	0.861
Range, pg/mL	0.69–0.69	0.69–5.22	0.68–3.42	0.69–4.24	
Above DV, %	0	4.5	7.4	4.6	
IL-18					
Median, pg/mL	5.22	5.36	5.48	5.36	0.102
Range, pg/mL	4.74–5.65	4.66–6.17	4.84–6.78	4.50–6.87	
Above DV, %	100	100	100	100	
TNF- α					
Median, pg/mL	2.46	3.58	3.06	2.97	0.102
Range, pg/mL	2.46–4.66	2.46–6.63	2.46–6.29	2.46–8.83	
Above DV, %	38.9	66.3	55.6	54.9	
IL-4					
Median, pg/mL	1.36	1.36	1.36	1.36	0.410
Range, pg/mL	1.36–1.36	1.36–4.39	1.36–3.50	1.36–6.14	
Above DV, %	0	4.5	11.1	7.2	
IL-5					
Median, pg/mL	0.69	0.69	0.69	0.69	0.343
Range, pg/mL	0.69–1.17	0.69–4.07	0.69–3.66	0.69–3.77	
Above DV, %	5.6	13.5	22.2	11.8	
IL-6					
Median, pg/mL	0.69	1.20	1.50	1.30	0.092
Range, pg/mL	0.69–5.34	0.69–10.09	0.69–6.65	0.69–5.34	
Above DV, %	5.6	23.6	37.0	22.2	
IL-9					
Median, pg/mL	3.42	3.42	3.42	3.42	0.210
Range, pg/mL	3.42–3.42	3.42–4.75	3.42–5.77	3.42–6.74	
Above DV, %	0	1.1	7.4	2.0	
IL-10					
Median, pg/mL	0.69	0.85	0.90	0.85	0.240
Range, pg/mL	0.69–2.22	0.69–3.45	0.69–2.54	0.69–3.52	
Above DV, %	27.8	53.9	55.6	56.2	

Table 1 (Continued).

Cytokine	Controls (<i>n</i> = 18)	AA subgroup			<i>P</i> value*
		AAP (<i>n</i> = 89)	AAT (<i>n</i> = 27)	AT/AU (<i>n</i> = 153)	
IL-13					
Median, pg/mL	3.67	3.67	3.67	3.67	0.554
Range, pg/mL	3.67–4.10	3.67–4.77	3.67–4.92	3.67–7.06	
Above DV, %	5.6	12.4	18.5	11.8	
IL-1ra					
Median, pg/mL	4.04	5.12	5.08	5.07	0.072
Range, pg/mL	2.05–5.95	–0.22–9.27	2.05–8.12	2.05–8.41	
Above DV, %	77.8	85.4	92.6	90.2	
IL-1 α					
Median, pg/mL	1.66	2.04	2.03	1.98	0.675
Range, pg/mL	0.69–3.43	0.69–5.61	0.69–4.51	0.69–4.87	
Above DV, %	66.7	78.6	66.7	82.4	
IL-1 β					
Median, pg/mL	0	0.09	0.68	0	0.081
Range, pg/mL	0–4.14	0–7.34	0–4.56	0–6.14	
Above DV, %	22.2	51.7	51.8	47.7	
IL-8					
Median, pg/mL	0.69	5.10	5.50	5.12	0.017
Range, pg/mL	0.69–11.11	0.69–13.17	0.69–11.35	0.69–13.25	
Above DV, %	33.3	71.9	59.3	75.8	
RANTES					
Median, pg/mL	10.84	11.22	11.03	11.06	0.122
Range, pg/mL	9.40–11.86	9.42–12.58	9.42–11.82	9.19–12.62	
Above DV, %	100	100	100	100	

AA, alopecia areata; AAP, persistent alopecia areata; AAT, transient alopecia areata; AT, alopecia totalis; AU, alopecia universalis; IFN, interferon; IL, interleukin; IL-ra, interleukin receptor antagonist; RANTES, regulated upon activation, normal T-cell expressed and secreted; TNF, tumour necrosis factor. *Kruskal–Wallis test; †above minimum detectable value (DV). Data are log transformed.

had no personal or family history of AA. Of the patients with AA, 44% reported having only one episode, 43% reported 2–10 episodes and 13% reported > 10. In total, 144 of the patients with AA (54%) and 8 (44%) of the 18 control subjects reported ≥ 1 form of atopy.

Overall, serum levels of the cytokines IFN- γ , IL-2, IL-12, IL-6 and IL-10 were greater in patients with AA than in unaffected controls but varied among patients with different AA phenotypes (Fig. 1). Median IFN- γ levels were higher in patients with in the AA subgroups compared with controls, with the greatest difference occurring in patients with AAT compared with controls (Table 1). When we examined the dispersion of cytokine values occurring above the minimum detectable value, median levels of IL-2 and IL-12 displayed the greatest differences in patients with AA and controls (Table 2). The median of the log transformation for IL-2 was 3.58 pg/mL in patients with AAT, 3.12 pg/mL in

patients with AAP and 2.94 pg/mL in patients with AT/AU, compared with 2.66 pg/mL in controls ($P = 0.042$). This suggests that there is a Th1 response early in the disease, as patients with AAT had higher serum IL-2 levels than patients with AT and AU, and both AA subgroups (patients with AAT and patients with AT and AU) had higher serum IL-2 levels than controls (Fig. 1).

Of the Th2 cytokines, IL-4 and IL-9 levels were below the minimum detectable value in most of the patients and IL-5 and IL-13 were measurable in only one patient's serum. Median serum levels of IL-8 ($P = 0.017$) were higher in all patients with AA than in controls. The differences in median serum levels of IL-1ra ($P = 0.072$) and IL-6 ($P = 0.09$) between AA and controls (Fig. 1) were of borderline significance.

When we stratified patients with AA by disease activity and number of AA episodes for the 17 cytokines, no

Table 2 Comparison of median dispersion above minimum detectable value of serum cytokines in alopecia areata subgroups and controls.

Cytokine	AA subgroup				P value*
	Controls (n = 18)	AAP (n = 89)	AAT (n = 27)	AT/AU (n = 153)	
IFN- γ					
n	11	74	20	130	
Median	1.86	2.29	2.52	2.24	0.198
Range	1.18–2.71	0.71–4.66	0.78–4.21	0.72–5.49	
IL-2					
n	10	64	18	108	
Median	2.66	3.12	3.58	2.94	0.042
Range	2.00–3.91	1.40–5.81	2.55–5.09	1.43–5.00	
IL-12					
n	6	49	14	79	
Median	1.64	2.22	2.52	1.92	0.006
Range	1.22–2.33	1.23–4.58	1.62–4.08	1.08–4.03	
IL-15					
n	0	4	2	7	
Median	–	1.83	2.12	2.51	0.773
Range	–	1.53–5.22	0.82–3.42	1.04–4.24	
IL-18					
n	18	89	27	153	
Median	5.22	5.36	5.48	5.36	0.103
Range	4.74–5.65	4.66–6.17	4.84–6.78	4.50–6.87	
TNF- α					
n	7	59	15	84	
Median	3.87	4.18	4.55	4.15	0.510
Range	3.08–4.66	2.51–6.63	2.58–6.29	2.48–8.83	
IL-4					
n	0	4	3	11	
Median	–	2.31	1.57	2.36	0.493
Range	–	1.60–4.39	1.48–3.50	1.43–6.14	
IL-5					
n	1	12	6	18	
Median	1.17	1.61	1.93	1.51	0.890
Range	1.17–1.17	0.74–4.07	0.77–3.66	0.78–3.77	
IL-6					
n	8	58	18	113	
Median	1.37	1.91	2.74	1.66	0.149
Range	0.76–5.34	0.70–10.09	1.02–6.65	0.70–9.43	
IL-9					
n	0	1	2	3	
Median	–	4.75	5.76	6.65	0.651
Range	–	4.75–4.75	5.74–5.77	4.14–6.74	
IL-10					
n	5	48	15	86	
Median	1.25	1.44	1.69	1.28	0.240

Table 2 (Continued).

Cytokine	AA subgroup				P value*
	Controls (n = 18)	AAP (n = 89)	AAT (n = 27)	AT/AU (n = 153)	
Range	0.82–2.22	0.76–3.45	0.73–2.54	0.70–3.52	
IL-13					
n	1	11	5	18	
Median	4.10	4.12	4.88	4.28	0.372
Range	4.10–4.10	3.74–4.77	3.84–4.92	3.72–7.06	
IL-1ra					
n	14	76	25	138	
Median	4.42	5.40	5.09	5.25	0.050
Range	2.94–5.95	2.77–9.27	2.08–8.12	2.40–8.41	
IL-1 α					
n	12	70	18	126	
Median	2.34	2.20	2.92	2.28	0.308
Range	0.91–3.43	0.69–5.61	0.92–4.51	0.72–4.87	
IL-1 β					
n	4	46	14	73	
Median	0.85	1.45	2.74	1.23	0.147
Range	0.54–4.14	0.09–7.34	0.68–4.56	0.03–6.14	
IL-8					
n	6	64	16	116	
Median	4.80	6.73	8.99	5.99	0.062
Range	1.70–11.11	1.54–13.17	2.80–11.35	1.12–13.25	
RANTES					
n	18	89	27	153	
Median	10.84	11.22	11.03	11.06	0.122
Range	9.40–11.87	9.42–12.58	9.42–11.82	9.19–12.62	

AA, alopecia areata; AAP, persistent alopecia areata; AAT, transient alopecia areata; AT, alopecia totalis; AU, alopecia universalis; IFN, interferon; IL, interleukin; IL-1ra, interleukin-1 receptor antagonist; RANTES, regulated upon activation, normal T-cell expressed and secreted; TNF, tumour necrosis factor. *Kruskal-Wallis test. Data are log transformed.

significant differences in serum cytokine levels were seen in patients with active disease ($n = 258$) compared with patients without active disease ($n = 8$) (data not shown). Median serum cytokine levels of IL-2, IL-6 and IL-8, but not IL-12, IFN- γ , IL-4, IL-10 and IL-1ra, were significantly different between the groups with different reported numbers of AA episodes. IL-2 was highest in patients with 2–10 episodes (2.87 pg/mL) compared with patients with 1 (2.49) or > 10 (2.77 pg/mL; $P = 0.04$) episodes. IL-6 levels were also highest in the group with 2–10 episodes (1.53 pg/mL; $P = 0.03$) compared with the groups with 1 (1.15 pg/mL) or 10 (0.87 pg/mL) episodes. IL-8 levels (5.73 pg/mL) were similarly highest in patients with 2–10 episodes, and

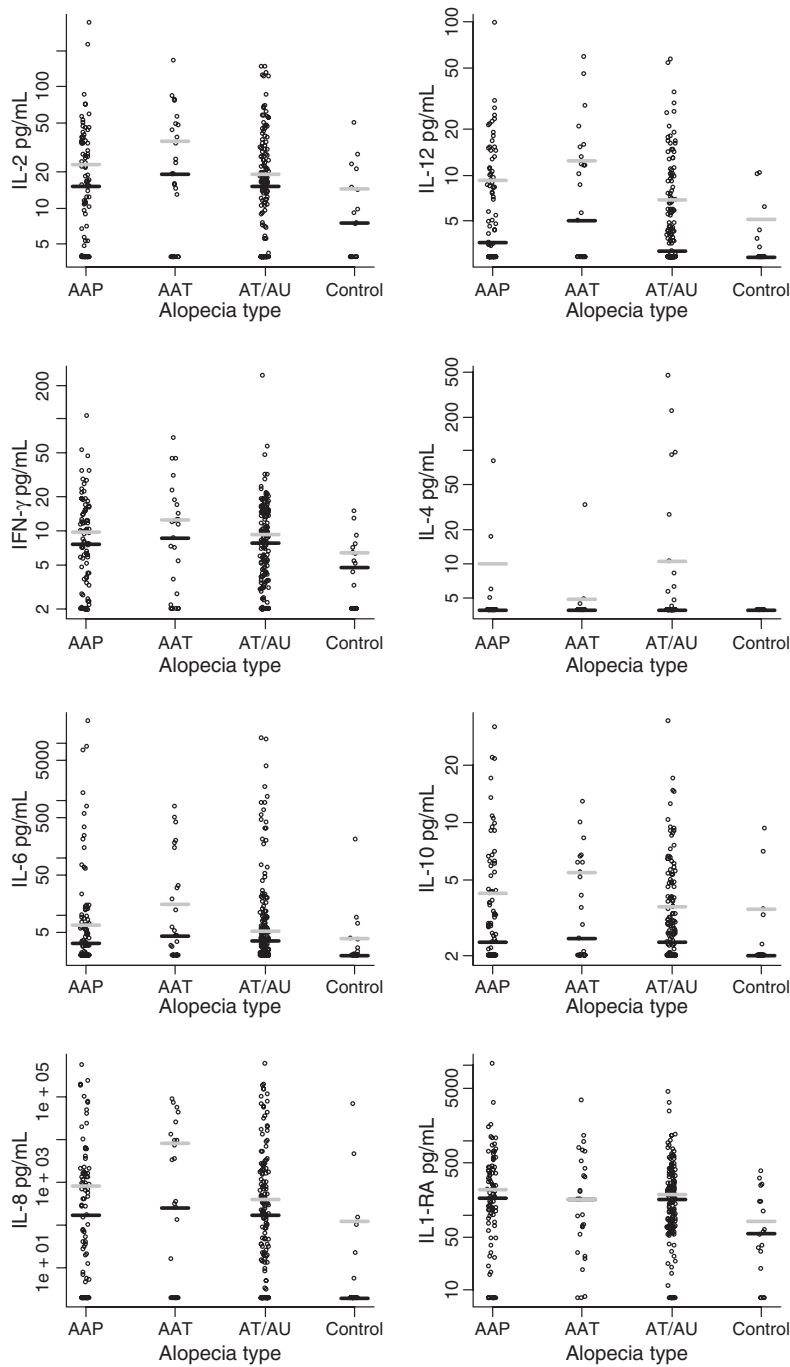


Figure 1 Distribution of cytokine levels for interleukin (IL)-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-1 receptor antagonist (ra) and interferon (IFN)- γ in sera (overall and above minimum detectable levels). Black line, overall median serum cytokine levels; grey line, median serum cytokine levels above the minimum detectable level. AA, alopecia areata; AAP, persistent alopecia areata; AAT, transient alopecia areata; AT, alopecia totalis; AU, alopecia universalis.

lower in the groups with 1 (4.61 pg/mL) and > 10 (2.74 pg/mL) episodes ($P = 0.045$) (Table S1).

The small number of controls with atopy (8 of 18) made comparisons uninformative (data not shown). Of the atopic patients with AA, 14 (10%) had AAT, 51 (35%) had AAP and 79 (55%) had AT/AU. When we compared serum cytokine levels in AA subjects with or without atopy, patients with atopy had higher median

serum cytokine levels of TNF- α (3.72 pg/mL vs. 2.46 pg/mL; $P = 0.008$), IL-1 α (2.15 pg/mL vs. 1.61 pg/mL; $P = 0.003$), IFN- γ (2.09 pg/mL vs. 1.87 pg/mL; $P = 0.077$) and IL-12 (1.43 pg/mL vs. 1.06 pg/mL; $P = 0.056$) (Table S2). There were no significant differences in median serum cytokine levels in AA subgroups with atopy compared with those without atopy (Table S3 online).

Discussion

A predominance of Th1 cytokines (IL-2, IL-12 and IFN- γ) has been found previously in AA skin lesions from patients with AA and C3H/HeJ AA mice,^{10–14} and in this study we also found that Th1 cytokines are increased in the sera of patients with AA. Two small studies have previously examined serum cytokine levels in AA in smaller numbers of subjects.^{10,14} Teraki *et al.* studied 14 patients (7 AAP and 7 AU) and found that the levels of IL-1 α and the Th2 cytokine IL-4 were significantly increased in samples from patients with localized AA and also that IFN- γ and IL-2 levels were significantly increased in samples from patients with AT/AU.¹⁰ Arca *et al.* found that IFN- γ levels were greater in a group of 40 patients with AA than in controls.¹⁴

Our study differs from those of Teraki *et al.* and Arca *et al.* by having many more subjects with a confirmed diagnosis of AA and by stratifying by the severity phenotypes, disease activity and associated atopy. In addition, we used a new multianalyte array that allowed us to examine 17 different cytokines simultaneously. In our study, the Th1 cytokines, IFN- γ , IL-2 and IL-12 levels were higher in the sera from all patients with AA than in the controls. Our study also confirms the findings of Teraki *et al.* and Arca *et al.* that levels of IFN- γ in patients with AA are higher than in controls.^{10,14} In addition, IL-4 was detected in a small percentage of the AA samples but was undetectable in controls. IL-6 and IL-10 levels were also greater in patients with AA than controls, but the difference was not significant.

We investigated whether different cytokine profiles correlated with phenotypic severity of AA, disease activity and associated atopy. IL-6 and IL-10 levels were higher in all patients with AA and also in the subgroup of patients with AAT than in controls. Teraki *et al.* found that levels of IL-4 were higher in patients with patchy AA than in controls,¹⁰ whereas we found that IL-4 levels were higher in patients with AT/AU than in those with AAT and were undetectable in controls. However, there were no differences in IL-4 levels among AT/AU and AAT with respect to their atopic status. We classified AAT and AAP according to NAAR definitions, whereas Teraki *et al.* combined patients with AAP and AAT into one group. In addition, the differences in IL-4 levels were seen among the dispersions (i.e. those values above minimum detectable value), but only a small percentage of the samples were above the minimum detectable value for each subgroup. It also should be noted that 55% of our subjects had

AT/AU, which reflects a selection bias because patients with a more severe phenotype are more likely to register and participate in studies.

Although the balance of Th1 and Th2 cytokines may play a role in AA, other cytokines may also be involved. In particular, we found that IL-1ra levels are higher in the sera of patients with AA than in the sera of control subjects, and to our knowledge, this is the first such report. We also found higher levels of IL-8 in patients with AA than in controls, confirming the findings of Kuwano *et al.* of increased serum levels of IL-8, RANTES and IFN- γ in patients with AA.¹⁶ We also found higher levels of IL-2, IL-6 and IL-8 in patients who had 2–10 episodes of AA compared with patients who had experienced only 1 episode or > 10 episodes. IL-8 is a chemokine secreted by keratinocytes that is overexpressed in psoriasis and AD.^{17,18} We have previously reported that fibroblasts from patients with psoriasis induce IL-8 overexpression and hyperproliferation in foreskin keratinocytes.¹⁸ We speculate that increased IL-8 cytokine levels in AA could play a role in perturbing keratinocyte differentiation in the AA follicle.

IL-1ra binds to the receptors for the pro-inflammatory cytokines, IL-1 α and IL-1 β , and blocks signal transduction.¹⁹ The balance between the levels of these two cytokines, their receptors and IL-1ra plays an important role in hair growth and differentiation.^{20,21} Both IL-1 α and IL-1 β inhibit hair growth and overexpression of IL-1 α results in patchy hair loss in mouse models.^{21,22} Alleles 1 and 2 of the receptor antagonist gene (*IL-1RN*) have previously been associated with AA,^{23,24} but the role of the receptor antagonist in AA is unclear.

Patients with AA and atopy were found to have higher levels of IL-1 α , TNF- α , IFN- γ and IL-12 than patients with AA without atopy. This finding was surprising because atopy is generally associated with Th2 cytokine predominance.⁹ This would suggest that serum cytokines related to AA are predominant over atopy in patients with AA.²⁵ A larger group of controls screened for atopy should be studied to explore this possibility.

In summary, our data support the hypothesis that Th1 serum cytokines, IL-8 and IL-1ra are associated with AA and may be involved in its pathogenesis.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Comparison of median serum cytokine levels and number of episodes of alopecia areata.

Table S2. Comparison of overall median serum cytokine levels in patients with AA and atopy versus those with AA without atopy.

Table S3. Comparison of overall median serum cytokine levels among AA subgroups with atopy versus AA subgroups without atopy.

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Supplementary Table 1 Comparison of median serum cytokine levels and number of episodes of alopecia areata

Cytokine	Number of episodes			P value*
	1 (n = 111)	2–10 (n = 107)	> 10 (n = 33)	
IFN- γ				
Median	2.08	2.02	1.87	0.635
Range	0.68–4.66	0.68–3.79	0.69–5.49	
IL-2				
Median	2.49	2.87	2.77	0.045
Range	1.36–5.81	1.36–5.09	1.36–5.00	
IL-12				
Median	1.06	1.41	1.06	0.662
Range	1.06–4.58	1.06–3.81	1.06–4.03	
IL-4				
Median	1.36	1.36	1.36	0.852
Range	1.36–4.57	1.36–5.42	1.36–6.14	
IL-6				
Median	1.15	1.53	0.87	0.027
Range	0.69–9.43	0.69–10.09	0.69–8.31	
IL-10				
Median	0.90	0.93	0.69	0.716
Range	0.69–3.08	0.69–3.45	0.69–3.52	
IL-1ra				
Median	4.98	5.30	4.72	0.198
Range	–0.22–9.27	2.05–8.07	2.05–8.41	
IL-8				
Median	4.61	5.73	2.74	0.045
Range	0.69–13.25	0.69–13.17	0.69–12.12	

IFN, interferon; IL, interleukin; IL-1ra, interleukin-1 receptor antagonist. *Kruskal–Wallis test. Data are log transformed

Supplementary Table 2 Comparison of overall median serum cytokine levels in patients with AA and atopy versus those with AA without atopy

Cytokine	AA subgroup		Pvalue ^a
	AA/atopy (n=144)	AA/no atopy (n=125)	
IFN- γ			
Median	2.09	1.87	0.077
Range	0.69–4.66	0.68–5.49	
IL-2			
Median	2.71	2.72	0.892
Range	1.36–5.4	1.36–5.81	
IL-12			
Median	1.43	1.06	0.056
Range	1.06–4.08	1.06–4.58	
IL-15			
Median	0.69	0.69	0.179
Range	0.69–4.24	0.68–5.22	
IL-18			
Median	5.37	5.36	0.754
Range	4.73–6.78	4.5–6.87	
TNF- α			
Median	3.72	2.46	0.008
Range	2.46–6.38	2.46–8.83	
IL-4			
Median	1.36	1.36	0.197
Range	1.36–5.42	1.36–6.14	
IL-5			
Median	0.69	0.69	0.571
Range	0.69–3.66	0.69–4.07	

IL-6			
Median	1.27	1.37	0.955
Range	0.69–10.09	0.69–9.43	
IL-9			
Median	3.42	3.42	0.847
Range	3.42–5.77	3.42–6.74	
IL-10			
Median	0.85	0.86	0.597
Range	0.69–3.52	0.69–3.08	
IL-13			
Median	3.67	3.67	0.398
Range	3.67–5.09	3.67–7.06	
IL-1ra			
Median	5.1	5.08	0.470
Range	–0.22–8.41	2.05–9.27	
IL-1 α			
Median	2.15	1.61	0.003
Range	0.69–4.9	0.69–5.61	
IL-1 β			
Median	0.11	0	0.698
Range	0–6.99	0–7.34	
IL-8			
Median	4.98	5.12	0.861
Range	0.69–13.17	0.69–13.25	
RANTES			
Median	11.12	11.11	0.617
Range	9.26–12.58	9.19–12.62	

AA, alopecia areata; IL, interleukin; RANTES, regulated upon activation, normal T-cell expressed and secreted; TNF, tumour necrosis factor. *Kruskal–Wallis test. Data are log transformed

Supplementary Table 3 Comparison of overall median serum cytokine levels among AA subgroups with atopy versus AA subgroups without atopy.

Cytokine	With atopy			<i>P</i> value*	Without atopy			<i>P</i> value*
	AAP (<i>n</i> = 51)	AAT (<i>n</i> = 14)	AT/AU (<i>n</i> = 79)		AAP (<i>n</i> = 38)	AAT (<i>n</i> = 13)	AT/AU (<i>n</i> = 74)	
IFN- γ								
Median	2.13	1.97	2.09	0.805	1.85	2.42	1.80	0.685
Range	0.69–4.66	0.69–4.21	0.69–3.46		0.68–3.53	0.69–3.79	0.68–5.49	
IL-2								
Median	2.70	2.90	2.75	0.992	2.71	2.95	2.63	0.403
Range	1.36–5.40	1.36–4.42	1.36–5.00		1.36–5.81	1.36–5.09	1.36–4.98	
IL-12								
Median	156	1.40	1.41	0.579	1.15	1.62	1.06	0.424
Range	1.06–3.42	1.06–4.08	1.06–4.03		1.06–4.58	1.06–3.81	1.06–3.54	
IL-15								
Median	0.69	0.69	0.69	0.433	0.69	0.69	0.69	0.154
Range	0.69–2.06	0.69–3.43	0.69–4.24		0.69–5.22	0.68–0.69	0.69–3.15	
IL-18								
Median	5.35	5.32	5.38	0.896	5.37	5.48	5.33	0.185
Range	4.73–6.17	4.84–6.78	4.78–6.48		4.66–5.92	5.10–5.92	4.50–6.87	
TNF- α								
Median	3.93	3.60	3.71	0.386	2.97	2.58	2.46	0.804
Range	2.46–6.38	2.46–6.00	2.46–6.07		2.46–6.63	2.46–6.29	2.46–8.83	
IL-4								
Median	1.36	1.36	1.36	0.477	1.36	1.36	1.36	0.745
Range	1.36–1.77	1.36–3.50	1.36–5.42		1.36–4.39	1.36–1.57	1.36–6.14	
IL-5								
Median	0.69	0.69	0.69	0.665	0.69	0.69	0.69	0.419
Range	0.69–3.29	0.69–3.66	0.69–3.18		0.69–4.07	0.69–2.37	0.69–3.77	
IL-6								
Median	1.09	1.26	1.32	0.669	1.48	1.68	1.17	0.524

Range	0.69–10.09	0.69–6.65	0.69–9.37		0.69–9.10	0.69–6.24	0.69–9.43	
IL-9								
Median	3.42	3.42	3.42	0.353	3.42	3.42	3.42	0.297
Range	3.42–4.75	3.42–5.77	3.42–4.15		3.42–3.42	3.42–5.74	3.42–6.74	
IL-10								
Median	0.78	0.71	0.94	0.932	0.93	1.06	0.78	0.582
Range	0.69–3.45	0.69–2.54	0.69–3.52		0.69–3.08	0.69–2.29	0.69–2.52	
IL-13								
Median	3.67	3.67	3.67	0.839	3.67	3.67	3.67	0.545
Range	3.67–4.77	3.67–4.88	3.67–5.09		3.67–4.75	3.67–4.92	3.67–7.06	
IL-1ra								
Median	5.12	4.43	5.19	0.805	5.19	5.33	5.03	0.708
Range	–0.22–7.32	2.05–8.12	2.05–8.41		2.05–9.27	2.05–6.67	2.05–8.04	
IL-1α								
Median	2.11	2.37	2.27	0.594	1.59	1.99	1.62	0.949
Range	0.69–4.90	0.69–4.19	0.69–4.62		0.69–5.61	0.69–4.51	0.69–4.87	
IL-1β								
Median	0.13	0.34	0.04	0.757	0.04	1.34	0.00	0.604
Range	0.00–6.99	0.00–4.56	0.00–5.66		0.00–7.34	0.00–3.95	0.00–6.14	
IL-8								
Median	4.37	3.83	5.24	0.911	5.60	5.73	4.56	0.856
Range	0.69–13.17	0.69–11.35	0.69–12.12		0.69–12.36	0.69–10.66	0.69–13.25	
RANTES								
Median	11.25	10.96	11.11	0.226	11.19	11.14	11.03	0.896
Range	9.42–12.58	10.48–11.43	9.26–12.27		9.84–12.17	9.42–11.82	9.19–12.62	

AA, alopecia areata; AAP, persistent alopecia areata; AAT, transient alopecia areata; AT, alopecia totalis; AU, alopecia universalis; IL, interleukin; RANTES, regulated upon activation, normal T-cell expressed and secreted; TNF, tumour necrosis factor. *Kruskal–Wallis test. Data are log transformed.