Reduced Expression of Interleukin-2 Decreases the Frequency of Alopecia Areata Onset in C3H/HeJ Mice

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Alopecia areata (AA) is an autoimmune hair loss disease, that can be transferred between C3H/HeJ mice by skin grafting. We explored whether AA susceptibility is influenced by the availability of interleukin (IL)-2, a cytokine with leukocyte activating and regulatory properties. Mice heterozygous for a targeted deletion of IL-2 from the histocompatible C3.129P2(B6)-*Il2*^{tm1Hor} substrain, that produce reduced levels of IL-2, were examined for AA development after grafting skin from AA-affected C3H/HeJ mice. After grafting, nine of 19 (47%) heterozygous IL-2$^{+/-}$ *versus* 16 of 18 (88%) IL-2$^{+/+}$ wild-type littermates developed AA. Although dense follicular leukocyte infiltrates were apparent in AA affected wild-type mice, AA-developing IL-2$^{+/-}$ littermates had a reduced leukocyte infiltration, and AA-resistant IL-2$^{+/-}$ mice had no inflammation. Lymph node cell analysis revealed a reduction in leukocyte activation markers in AA-developing IL-2$^{+/-}$ mice. IL-2$^{+/-}$ mice presented with low level expression of cytokines (IL-4, IL-10, interferon-γ, transforming growth factor-β), upregulation of tumor necrosis factor receptors, and increased leukocyte apoptosis susceptibility independent of AA expression. In the skin, CD4^{+} cells and monocytes were reduced; activation markers were not upregulated and very few CD44v3^{+} or CD44v10^{+} leukocytes were recovered. Taken together, our data suggest that AA resistance of IL-2$^{+/-}$ mice is because of the failure of activated leukocyte recruitment, thus pointing toward an involvement of IL-2 in AA pathogenesis.

Key words: alopecia areata/experimental mouse model/interleukin-2/T-lymphocytes
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Alopecia areata (AA) is a putative autoimmune disease of anagen stage hair follicles (McElwee and Hoffmann, 2002; Paus *et al*, 2003). In the C3H/HeJ mouse model, AA can be transferred from spontaneous AA-affected mice to normal haired littermates by full thickness skin grafts (McElwee *et al*, 1998). Transfer experiments showed that CD4^{+} T helper cells (TH) are essential for disease transfer, and AA onset was restrained by regulatory T cells (McElwee *et al*, 2005). The cytokine profile of AA-affected mice reveals both TH-1 and TH-2 are upregulated (McElwee *et al*, 2002). Because of the requirement for TH, the generalized upregulation of cytokines, and the fact that the clinical AA phenotype is restricted to hair follicles and other skin appendages (McElwee *et al*, 2001) we suspected that a reduction in TH/TH factors would reduce the disease incidence as a consequence of insufficient autoreactive T cell activation.

We examined the role of interleukin (IL)-2 in AA using IL-2$^{+/-}$ mice. IL-2 stimulates lymphocyte proliferation and cytokine production (Paul, 1989), but also exerts regulatory functions by promoting regulatory T cells (Malek, 2003) and by increasing apoptosis susceptibility (Banz *et al*, 2002). Homozygous IL-2 knockout mice are characterized by anemia, lymphoproliferative disorders and colitis (Horak, 1995; Nelson, 2002; Schimpl *et al*, 2002). Because IL-2$^{-/-}$ mice have a high mortality rate *in utero* and have a significantly curtailed life-span (Sadlack *et al*, 1993), our study was necessarily limited to IL-2$^{+/-}$ mice. Heterozygous IL-2$^{+/-}$ mice appear to have overtly normal hematopoiesis and immune response regulation (Paul, 1989). Due to the monoallelic expression of IL-2, however, they display reduced IL-2 levels (Hollander *et al*, 1998) and are well suited to explore the impact of IL-2 availability on immune response after full thickness skin grafts from AA-affected mice. All animal experiments were approved by the local animal care authorities (Giessen, Germany).

Heterozygous C3.129P2(B6)-*Il2*^{tm1Hor} (IL-2$^{+/-}$ C3H/HeJ) mice appeared overtly healthy with normal weight and unaltered life expectation. Age- and sex-matched IL-2$^{+/+}$ or IL-2$^{+/-}$ mice (6 months old) received full thickness skin transplants of AA-affected C3H/HeJ mice (McElwee *et al*, 2003). Within 6–7 wk after skin grafting, 16 of 18 (88%) IL-2$^{+/+}$, but only 9/19 (47%) IL-2$^{+/-}$ mice (p 0.013) developed hair loss. Immunohistochemistry of skin revealed a dense perifollicular and intrafollicular infiltrate of CD8^{+} cells and a moderate perifollicular infiltrate of CD4^{+} cells in

Abbreviations: AA, alopecia areata; IFN, interferon; IL, interleukin; LNC, lymph node cells; Mϕ, monocytes; R, receptor; SkIL, skin infiltrating leukocytes; TGF, transforming growth factor; TH, T helper cells; TNF, tumor necrosis factor

[1]Equal contributions.

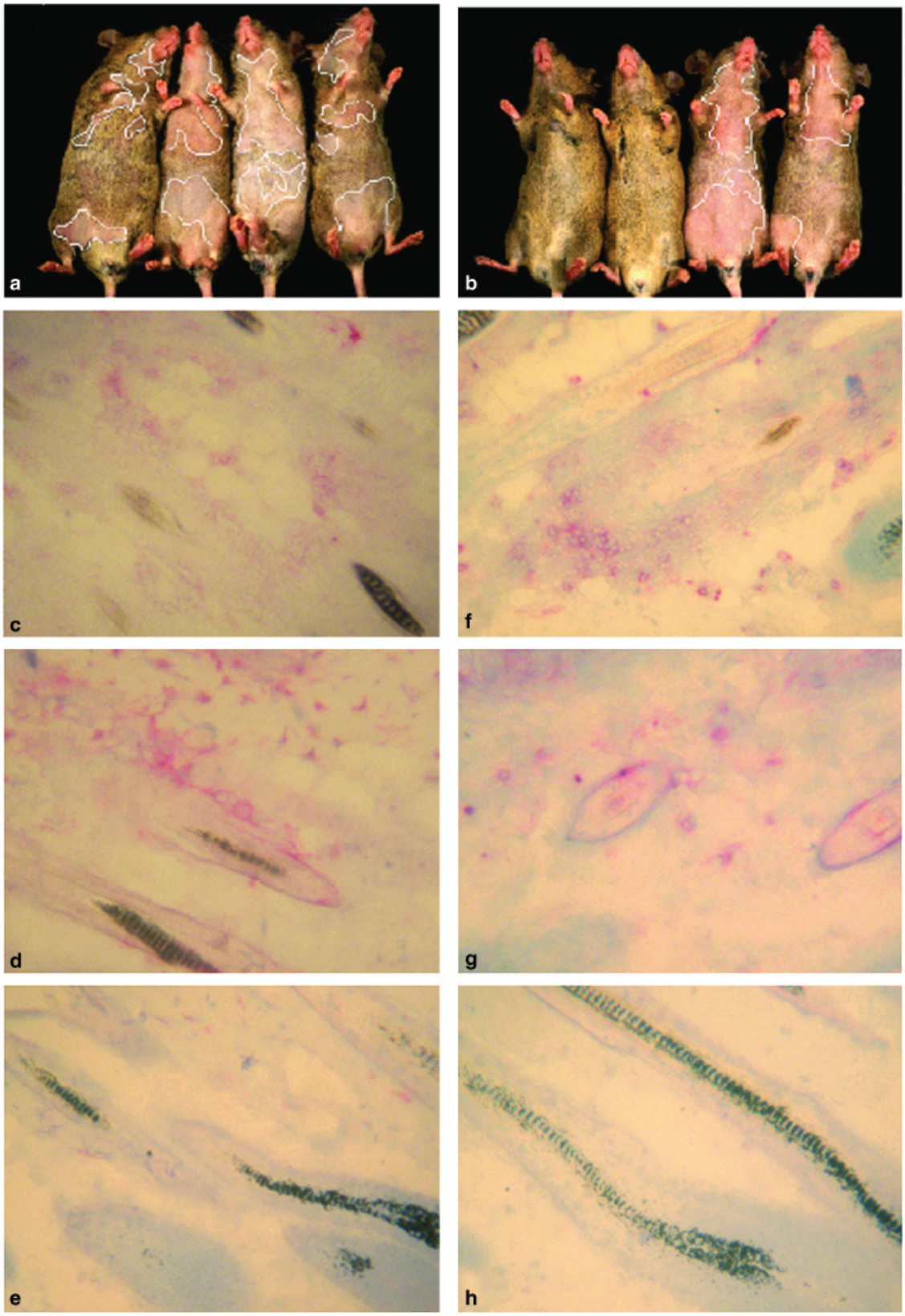

Figure 1
Interleukin (IL)-2$^{+/-}$ mice are relative resistant to alopecia areata (AA) development. At 8 wk after induction of AA by grafting lesional AA-skin from C3H/HeJ mice onto the back of the recipients (for details, see addendum), 88% of IL-2$^{+/+}$ mice (*a*), but only 47% of IL-2$^{+/-}$ mice (*b*) developed AA. The ventral surface of four IL-2$^{+/+}$ mice with AA (*a*) and of 4 IL-2$^{+/-}$ mice, two of which developed AA (*b*) is shown. Immunohistochemical staining (for details, see addendum) for CD4 (*c–e*) and CD8 (*f–h*) of frozen skin sections of IL-2$^{+/+}$ mice with AA (*c*,*f*), IL-2$^{+/-}$ mice with AA (*d*,*g*) and IL-2$^{+/-}$ mice without AA (*e*, *i*, *h*) is shown. Moderate perifollicular infiltrates of CD4^{+} cells were identified in IL-2$^{+/+}$ mice (*c*) and IL-2$^{+/-}$ mice with AA (*d*), dense peri- and intrafollicular infiltrates of CD8^{+} cells were seen in IL-2$^{+/+}$ mice with AA (*f*) and moderate CD8^{+} cell infiltrates were apparent in IL-2$^{+/-}$ mice with AA (*g*). No perifollicular or intrafollicular infiltrates of CD4^{+} (*e*) or CD8^{+} (*h*) cells were observed in AA-resistant IL-2$^{+/-}$ mice.

Table I. IL-2 levels and impact of reduced IL-2 level on leukocyte activation in AA mice

Source of IL-2[b]	IL-2 ELISA (OD = 450)[a]		
	IL-2$^{+/+}$	IL-2$^{+/-}$	p value
(A) IL-2 Levels in IL-2$^{+/+}$ and IL-2$^{+/-}$ mice			
Serum (1:100)	0.606 ± 0.077	0.313 ± 0.039	0.0043
SC supernatant (PHA)	0.177 ± 0.038	0.103 ± 0.017	0.0391
SC supernatant (anti-CD3)	0.269 ± 0.024	0.155 ± 0.024	0.0044
LNC supernatant (PHA)	0.198 ± 0.024	0.137 ± 0.001	0.0120
LNC supernatant (anti-CD3)	0.280 ± 0.021	0.179 ± 0.021	0.0048

	Cell number (× 10^6)[a,c]										
Organ	IL-2$^{+/+}$	IL-2$^{+/-}$	IL-2$^{+/-}$: IL-2$^{+/+}$	IL-2$^{+/+}$ AA	IL-2$^{+/+}$ AA: IL-2$^{+/+}$	IL-2$^{+/-}$ AA	IL-2$^{+/-}$ AA: IL-2$^{+/-}$	IL-2$^{+/-}$ AA: IL-2$^{+/+}$ AA	IL-2$^{+/-}$ AA resistant	IL-2$^{+/-}$ AA resistant: IL-2$^{+/-}$	IL-2$^{+/-}$ AA resistant: IL-2$^{+/-}$ AA
(B) Cell number											
Spleen	85.2	75.3	–	110.4	+ +	97.2	–	+	82.1	–	+
Lymph nodes	24.6	29.8	–	69.4	+ + +	65.3	+ + +	–	31.5	–	+ + +
Skin	3.4	3.7	–	11.3	+ + +	7.9	+ +	+ +	4.1	–	+ +

	% of SkIL leukocyte subsets[a,c]										
Subpopulation	IL-2$^{+/+}$	IL-2$^{+/-}$	IL-2$^{+/-}$: IL-2$^{+/+}$	IL-2$^{+/+}$ AA	IL-2$^{+/+}$ AA: IL-2$^{+/+}$	IL-2$^{+/-}$ AA	IL-2$^{+/-}$ AA: IL-2$^{+/-}$	IL-2$^{+/-}$ AA: IL-2$^{+/+}$ AA	IL-2$^{+/-}$ AA resistant	IL-2$^{+/-}$ AA resistant: IL-2$^{+/-}$	IL-2$^{+/-}$ AA resistant: IL-2$^{+/-}$ AA
(C) Subpopulations of SkIL											
CD4	22.9	19.8	–	25.6	–	16.4	–	+	17.8	–	–
CD8	20.8	23.6	–	21.4	–	23.8	–	–	22.7	–	–
SIgM	13.7	15.7	–	16.1	–	17.4	–	–	16.4	–	–
CD11b (Mϕ)	11.4	10.9	–	17.6	+ +	9.3	–	+ +	9.9	–	–
CD16 (NK)	16.3	19.6	–	17.7	–	18.6	–	–	18.2	–	–

Table I. Continued

Marker	% stained LNC[a,c]								% stained SkIL[a,c]							
	IL-2$^{+/+}$	IL-2$^{+/+}$ AA	IL-2$^{+/+}$ AA: IL-2$^{+/+}$	IL-2$^{+/-}$ AA	IL-2$^{+/-}$ AA: IL-2$^{+/+}$ AA	IL-2$^{+/-}$ AA resistant	IL-2$^{+/-}$ AA resistant: IL-2$^{+/+}$ AA	IL-2$^{+/-}$ AA resistant: IL-2$^{+/-}$ AA	IL-2$^{+/+}$	IL-2$^{+/+}$ AA	IL-2$^{+/+}$ AA: IL-2$^{+/+}$	IL-2$^{+/-}$ AA	IL-2$^{+/-}$ AA: IL-2$^{+/+}$ AA	IL-2$^{+/-}$ AA resistant	IL-2$^{+/-}$ AA resistant: IL-2$^{+/+}$ AA	IL-2$^{+/-}$ AA resistant: IL-2$^{+/-}$ AA
(D) Leukocyte activation markers and co-stimulatory molecules																
CD25	19.2	34.2	+ + +	22.0	+ + +	18.1	+ + +	–	10.8	10.9	–	9.2	–	6.7	–	–
CD28	58.8	71.9	+ +	55.9	+ +	51.4	+ + +	–	6.4	18.5	+ + +	5.2	+ + +	5.9	+ + +	–
CD69	28.4	40.4	+ + +	39.3	–	29.1	+ + +	+ + +	11.5	16.5	+	8.7	+ +	10.9	+	–
CD152	7.8	9.8	–	10.7	–	8.7	–	–	6.7	8.1	–	10.2	–	8.7	–	–
CD154	6.9	15.2	+ + +	6.8	+ + +	3.9	+ + +	–	6.0	20.9	+ + +	3.6	+ + +	2.7	+ + +	–
CD40	8.8	11.3	+	7.7	+ +	7.6	+ +	–	11.3	19.9	+ + +	9.7	+ + +	6.2	+ + +	–
CD80	7.9	41.1	+ + +	21.6	+ + +	10.3	+ + +	+ + +	5.3	19.2	+ + +	15.7	–	14.5	+	–
CD86	27.1	48.3	+ + +	35.7	+ +	25.7	+ + +	+ +	8.9	19.3	–	10.3	–	8.1	–	–

Marker	% of stained SkIL[a,c]									
	IL-2$^{+/+}$	IL-2$^{+/-}$	IL-2$^{+/-}$: IL-2$^{+/+}$	IL-2$^{+/+}$ AA	IL-2$^{+/+}$ AA: IL-2$^{+/+}$	IL-2$^{+/-}$ AA	IL-2$^{+/-}$ AA: IL-2$^{+/-}$	IL-2$^{+/-}$ AA: IL-2$^{+/+}$ AA	IL-2$^{+/-}$ AA resistant	IL-2$^{+/-}$ AA resistant: IL-2$^{+/-}$	IL-2$^{+/-}$ AA resistant: IL-2$^{+/-}$ AA
(E) Leukocyte migration (CD44 standard and variants)											
CD44s	37.5	25.9	+ + +	36.7	–	26.9	–	+ + +	25.8	–	–
CD44v6	5.8	2.4	+ +	19.9	+ + +	10.6	+ + +	+ + +	5.4	+ +	+ + +
CD44v3	15.6	6.9	+ + +	23.9	+ + +	8.8	–	+ + +	7.4	–	–
CD44v10	9.8	3.9	+ + +	20.4	+ + +	9.5	+ + +	+ + +	7.2	+ +	–

Marker	% stained LNC[a,b,c]								% Stained SkIL[a,b,c]							
	IL-2$^{+/+}$	IL-2$^{+/+}$ AA	IL-2$^{+/+}$ AA: IL-2$^{+/+}$	IL-2$^{+/-}$ AA	IL-2$^{+/-}$ AA: IL-2$^{+/+}$ AA	IL-2$^{+/-}$ AA resistant	IL-2$^{+/-}$ AA resistant: IL-2$^{+/+}$ AA	IL-2$^{+/-}$ AA resistant: IL-2$^{+/-}$ AA	IL-2$^{+/+}$	IL-2$^{+/+}$ AA	IL-2$^{+/+}$ AA: IL-2$^{+/+}$	IL-2$^{+/-}$ AA	IL-2$^{+/-}$ AA: IL-2$^{+/+}$ AA	IL-2$^{+/-}$ AA resistant	IL-2$^{+/-}$ AA resistant: IL-2$^{+/+}$ AA	IL-2$^{+/-}$ AA resistant: IL-2$^{+/-}$ AA
(F) Cytokine expression																
IL-2	18.1	22.3	+ +	15.4	+ + +	14.1	+ + +	–	12.2	16.8	+ + +	12.7	+ + +	9.3	+ + +	+ + +
IL-4	35.9	43.7	+ + +	24.6	+ + +	23.2	+ + +	–	22.3	23.1	–	16.8	–	15.1	+ + +	–
IL-10	21.4	20.7	–	17.2	+ +	15.4	+ + +	+	11.6	16.3	+ +	12.4	+ +	11.5	+ + +	–
IL-12	23.6	28.1	+	24.1	+	20.7	+ + +	+	16.3	18.6	–	16.0	+	14.1	+ +	–
IFNγ	22.5	23.5	–	16.0	+ + +	14.7	+ + +	–	14.2	18.4	+ +	10.9	+ + +	10.1	+ + +	–

TNFα	27.9	33.1	+	27.5	+	19.2	+++	++	14.5	18.1	++	10.0	+++	10.1	+++	−		
TGFβ	19.9	20.1	−	11.5	++	9.4	+++	+	11.5	16.2	+	11.0	+	10.4	++	−		
	% stained LNC[a,b,c]								% stained SkIL[a,b,c]									
Marker	**IL-2$^{+/+}$**	**IL-2$^{+/+}$ AA**	**IL-2$^{+/+}$ AA: IL-2$^{+/+}$**	**IL-2$^{+/-}$ AA**	**IL-2$^{+/-}$ AA: IL-2$^{+/+}$ AA**	**IL-2$^{+/-}$ AA resistant**	**IL-2$^{+/-}$ AA resistant: IL-2$^{+/+}$ AA**	**IL-2$^{+/-}$ AA resistant: IL-2$^{+/-}$ AA**	**IL-2$^{+/+}$**	**IL-2$^{+/+}$ AA**	**IL-2$^{+/+}$ AA: IL-2$^{+/+}$**	**IL-2$^{+/-}$ AA**	**IL-2$^{+/-}$ AA: IL-2$^{+/+}$ AA**	**IL-2$^{+/-}$ AA resistant**	**IL-2$^{+/-}$ AA resistant: IL-2$^{+/+}$ AA**	**IL-2$^{+/-}$ AA resistant: IL-2$^{+/-}$ AA**		
(G) Death receptor ligands and apoptosis																		
CD95L	5.3	7.2	−	12.2	++	10.5	+	−	8.8	11.3	−	17.2	+++	11.4	−	+++		
CD120a	9.8	18.6	++	31.6	+++	16.9	−	+++	2.5	5.1	−	20.2	+++	18.4	+++	−		
CD120b	3.2	11.7	+++	13.7	−	8.6	++	+++	3.4	3.5	−	9.7	+++	5.1	−	++		
Annexin V	15.0	10.1	−	19.5	++	24.0	+++	−	25.3	22.6	−	36.3	+++	40.3	+++	−		

[a]The mean of three independent experiments is shown. For clarity of presentation, SD are only shown for the IL-2 cytokine ELISA.
[b]Supernatants of SC and LNC culture, cytokine expression and Annexin V staining were tested after *in vitro* stimulation as described in the addendum.
[c]Significance was calculated by the two-tailed Student's *t* test. p values were adjusted for multiple comparisons by the stepdown Bonferoni method of Holm (1979). p values <0.05, +; <0.01, ++; <0.001, +++.
AA, alopecia areata; IFN, interferon; IL, interleukin; LNC, lymph node cells; Mϕ, monocytes; SkIL, skin infiltrating leukocytes; TGF, transforming growth factor; TNF, tumor necrosis factor.

IL-2$^{+/+}$ mice with AA development. AA-affected IL-2$^{+/-}$ mice showed only moderate infiltrates of CD4$^+$ and CD8$^+$ cells. AA-resistant IL-2$^{+/-}$ mice did not show CD4$^+$ and CD8$^+$ cell infiltrates (Fig 1).

Serum levels of IL-2 were reduced and supernatants of spleen cell (SC) and lymph node cell (LNC) cultures of IL-2$^{+/-}$ C3H/HeJ mice contained less IL-2 than supernatants of IL-2 competent mice (Table IA). The number of lymphoid cells in spleen, draining lymph nodes and skin did not differ significantly in non-manipulated IL-2$^{+/+}$ and IL-2$^{+/-}$ mice. After skin transplantation, however, SC and skin infiltrating leukocytes (SkIL) expanded less efficiently in IL-2$^{+/-}$ than in IL-2$^{+/+}$ AA developing mice. Lymphocytes did not expand at all in AA-resistant IL-2$^{+/-}$ mice (Table IB). In spleen and lymph nodes, the distribution between the major lymphocyte subsets (CD4$^+$-, CD8$^+$-, B-, NK-cells, and monocytes) did not differ significantly between IL-2$^{+/-}$ and IL-2$^{+/+}$ mice. This was independent of whether the mice did or did not develop AA (data not shown). The number of CD4$^+$ and of CD11b$^+$ SkIL, however, was reduced in IL-2$^{+/-}$ mice with AA as compared to IL-2$^{+/+}$ mice with AA (Table IC). Also, activation markers were increased in LNC and SkIL of IL-2$^{+/+}$ AA mice, but not in IL-2$^{+/-}$ AA mice. There were two exceptions, CD152 was not increased in IL-2$^{+/+}$ and IL-2$^{+/-}$ AA mice. Notably, too, CD25 expression was not increased in SkIL of IL-2$^{+/+}$ AA mice and a similar percentage of CD25$^+$ cells was seen IL-2$^{+/-}$ AA mice. In SkIL of AA-resistant IL-2$^{+/-}$ mice a further reduction in CD25$^+$ cells was noted. Upregulation of co-stimulatory molecule expression was also much stronger in LNC and SkIL of IL-2$^{+/+}$ than of IL-2$^{+/-}$ AA mice. Only CD80, the preferential CD152 ligand (Sansom *et al*, 2003), was expressed on a similar percentage of SkIL from IL-2$^{+/-}$ and IL-2$^{+/+}$ AA mice. Activation marker and co-stimulatory molecule expression did not significantly differ between IL-2$^{+/-}$ AA mice and IL-2$^{+/-}$ mice not developing AA (Table ID) further suggesting an impaired leukocyte activation in IL-2$^{+/-}$ mice, although expression of the regulatory T cell markers CD25 and CD152 was largely unaffected.

CD44s is involved in leukocyte activation and extravasation (Rafi-Janajreh *et al*, 1998; Siegelman *et al*, 2000). CD44v6 is primarily an activation marker (Marhaba *et al*, 2005). CD44v3 and CD44v10 are homing markers, particularly for SkIL (Rösel *et al*, 1997; Seiter *et al*, 1999; Freyschmidt-Paul *et al*, 2002). Expression of CD44s, CD44v3, CD44v10 and CD44v6 was unaltered in LNC (data not shown), but was reduced in SkIL of IL-2$^{+/-}$ mice. CD44 expression remained reduced in AA-affected IL-2$^{+/-}$ mice and particularly the reduction in CD44v6 expression became more striking in AA-affected IL-2$^{+/-}$ mice (Table IE), where, in addition, LNC CD44v6 expression was reduced (data not shown). The reduced recovery of CD44v6 likely reflects a reduced responsiveness of the IL-2$^{+/-}$ lymphocytes. In addition, reduced CD44 expression and, as a consequence a weakness in leukocyte extravasation (Carlow *et al*, 2001), obvioulsy hampers AA induction as apparent by the low incidence of AA in IL-2$^{+/-}$ mice after the transfer of AA-affected skin and the mild perifollicular infiltrate in the few mice developing AA.

Expression of the cytokines IL-2, IL-4, IL-10, IL-12, interferon (IFN)γ, tumor necrosis factor (TNF)α, and trans-

forming growth factor (TGF)β was reduced in LNC and SkIL of IL-2$^{+/-}$ as compared to IL-2$^{+/+}$ mice (data not shown). Because IL-2 stimulates the production of TH-1 and TH-2 cytokines (Paul, 1989), it is not surprising that expression of both regulatory and proinflammatory cytokines was reduced in AA-affected IL-2$^{+/-}$ as compared to IL-2$^{+/+}$AA mice. The fact the IL-12, TNFα and TGFβ, all three cytokines being preferentially expressed by activated Mϕ, was further reduced in draining LNC of AA-resistant IL-2$^{+/-}$ mice is consistent with a general deficit in TH activation, i.e. the reduced expression may reflect the reduced availability of IL-2 and as a consequence a reduction in Mϕ activation (Table IF).

CD95L and the death receptors CD120a and CD120b were all upregulated in LNC and SkIL of IL-2$^{+/-}$ as compared to IL-2$^{+/+}$ mice (data not shown) and remained elevated in IL-2$^{+/-}$ mice, which developed AA. Upregulation of death receptor expression was accompanied by an increase in apoptosis susceptibility as determined by AnnexinV-FITC staining after overnight culture in the presence of subthreshold levels of anti-CD3 (Table IG). It is generally accepted that IL-2 is involved in the maintenance of lymphocyte homeostasis by supporting activation induced cell death via upregulation of death receptors, their ligands and caspase 8 activation, or via passive apoptosis by the withdrawal of IL-2 after cessation of antigen and IL-2 stimulation (Lenardo, 1991; Kneitz *et al*, 1995; Refaeli *et al*, 1999). Though the latter could be pronounced in IL-2$^{+/-}$ mice, passive cell death has been shown to be mostly independent of death receptors and to proceed via downregulation of anti-apoptotic proteins (Nelson, 2002; Schimpl *et al*, 2002). Independent of these alternative death pathways, there is obviously enough IL-2 available in IL-2$^{+/-}$ mice to enable activation-induced cell death (AICD) as the levels of death receptors and apoptosis susceptibility were increased in AA-affected IL-2$^{+/-}$ mice. Accordingly, undue prevention of AICD by a relative shortage in IL-2 may not be of major importance for AA induction. Considering death receptor expression in AA-resistant IL-2$^{+/-}$ mice, these were mostly reduced as compared to AA-affected IL-2$^{+/-}$ mice, which we interpret as a secondary phenomenon in the sense, that T cell activation has not taken place. However, even in AA-resistant IL-2$^{+/-}$ mice apoptosis susceptibility remained elevated. Therefore, we suggest that quiescent T cell survival may be reduced in IL-2$^{+/-}$ mice (Lenardo, 1991). Whether such an overshooting death of naïve/resting T cells indirectly contributes towards AA resistance remains to be explored.

Taken together, a relative deficit in IL-2 did not promote AA, but rather it hampered AA development. The incidence of AA development after the transfer of AA-affected skin was significantly reduced and even those IL-2$^{+/-}$ mice that did develop AA displayed mild features of AA as compared to IL-2$^{+/+}$ mice. What does this tell us about AA induction? Taking the main features of IL-2, activation of TH, supporting AICD and maintaining the balance in regulatory T cells, our data suggest that the activation of TH is the most important event for AA induction. The expansion of CD4^{+} T cells was reduced, upregulation of activation markers was impaired, Mϕ activation, expression of costimulatory molecules and cytokine secretion by activated Mϕ was hampered and leukocyte migration (reduced CD44 expression) was affected.

IL-2 is also important for maintaining the balance in regulatory T cells, and a reduction in IL-2 could be a prominent feature of AA induction. In this case we would have expected an overshooting lymphoproliferation and an overall increase in the incidence and severity of AA in IL-2$^{+/-}$ mice. This was not the case. The third possibility whereby a relative shortage in IL-2 could affect AA induction relies on its involvement in lymphocyte homeostasis. IL-2 supports AICD, but IL-2 is also involved in the prevention of downregulation of anti-apoptotic proteins and can support resting T cell survival. These opposing features in the regulation of T cell survival, depending on their activation state, make it difficult to evaluate the impact on T cell survival in view of the relative AA resistance of IL-2$^{+/-}$ mice. Because AICD was not impaired, however, i.e., death receptors were upregulated and T cells, including T cells of AA-resistant IL-2$^{+/-}$ mice, were adequately driven into apoptosis it is obvious that a disbalance in AICD is not of major importance during the period of AA induction. Thus, without exception, the features of reduced TH responsiveness remained or were strengthened in IL-2$^{+/-}$ as compared to IL-2$^{+/+}$ AA mice. We interpret these features that during AA induction regulatory functions of IL-2 are probably less important than its role in TH activation.

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Supplementary Material

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Materials and Methods.

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