Immunogenetics (1999) 49:134–141

ORIGINAL PAPER

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The production of two Th2 cytokines, interleukin-4 and interleukin-10, is controlled independently by locus *Cypr1* and by loci *Cypr2* and *Cypr3*, respectively

Received: 2 March 1998 / Revised: 8 June 1998

Abstract The strains BALB/cHeA (BALB/c) and STS/A (STS) differ in production of IL-4 and IL-10, two Th2 cytokines, after stimulation of spleen cells with Concanavalin A, STS being a low and BALB/c a high producer. We analyzed the genetic basis of this strain difference using the recombinant congenic (RC) strains of the BALB/c-c-STS/Dem (CcS/Dem) series. This series comprises 20 homozygous strains. Each CcS/Dem strain contains a different, random set of approximately 12.5% genes of the "donor" strain STS and approximately 87.5% of the "background" strain BALB/c. We selected for further analysis the RC strain production intermediate between BALB/c and STS. In (CcS-20 × BALB/c)F_2 hybrids we found that different loci control expression of IL-4 and IL-10. *Cypr1* (cytokine production 1) on chromosome 16 near *D16Mit15* controls IL-4 production, whereas the production of IL-10 is influenced by loci *Cypr2* near *D1Mit14* and *D1Mit227* on chromosome 1 and *Cypr3* marked by *D5Mit20* on chromosome 5. In addition, the relationship between the level of these two cytokines depends on the genotype of the F_2 hybrids at a locus *cora1* (correlation 1) on chromosome 5. This differential genetic regulation may be relevant for the understanding of biological effects of T-helper cells in mice of different genotypes.

Key words Interleukin-4 · Interleukin-10 · Production · Gene · Regulation

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Introduction

Cytokine production is known to be influenced by the nature and amount of the antigen manner of presentation (Weaver et al. 1988), the presenting class II major histocompatibility complex (MHC) molecules (Murray et al. 1989), hormones (Almawi et al. 1996), and by the cytokines themselves (Mosmann and Sad 1996). Regulation may occur at the transcription level during secretion and circulation of the peptides and at the level of cytokine-target cell interaction. Mouse strains differ considerably in their ability to respond to mitogenic and antigenic challenge by cytokine production (Holáň et al. 1996; Raj et al. 1992), indicating an important role of the genotype in this process. Some loci influencing these processes have been described. It was shown that the *Ifl* locus on distal end of chromosome 3 (Mobraaten et al. 1984) controls the level of IFNα and IFNβ in different organs and in serum of mice infected by Newcastle disease virus (Raj et al. 1992); *Tms1* on the central part of chromosome 11 controls IFNγ expression after in vitro stimulation with IL-12 (Gorham et al. 1996). However, the most of the factors underlying genetic polymorphism in cytokine production remain unknown.

An imbalance in cytokine expression has been observed in atopic (Piccinni et al. 1996) and in autoimmune diseases (Charlton and Lafferty 1995), viral infections including HIV (Biron 1994), parasitic diseases (Sher and Coffman 1992), and in some cancers (Preisler et al. 1997; Strieter et al. 1995).

In some of these pathological reactions the Th2 cytokines interleukin-4 (IL-4) and IL-10 play an important role. These cytokines are pluripotent with an array of biological effects on several cell lineages. IL-4 has a major role in B-cell activation and isotype switching. It supports further production of IL-4 by T cells and plays an important role in determining the differentiation of naive $CD4^+$ T cells (Paul 1991). IL-10 is a potent suppressor of the effector functions of macrophages, T

cells, and NK cells. IL-10 also regulates proliferation and differentiation of B cells, mast cells, and thymocytes (Moore et al. 1993). To study genes which influence IL-4 and IL-10 expression, we used the recombinant congenic strains (RCS) of the BALB/c-c-STS/Dem (CcS/Dem) series, which were developed for genetic analysis of multigenically controlled biological processes (Demant 1992; Demant and Hart 1986). A series of RCS comprises 20 homozygous strains all produced by backcrossing and inbreeding from two parental inbred strains: a "background" strain and a "donor" strain. Each CcS/Dem strain of the series contains a different, random set of approximately 12.5% genes from the common donor strain STS and approximately 87.5% genes of the common background strain BALB/c. The RCS have been successfully used to study the genetics of susceptibility to colon (Moen et al. 1991; van Wezel et al. 1996) and lung (Fijneman et al. 1996) tumors, and activation of T lymphocytes (Havelková et al. 1996; Holáňet al. 1996; Krulová et al. 1997; Lipoldová et al. 1995), and may be especially useful in an analysis of relationships of different components of complex traits, such as susceptibility to infection (Demant et al. 1996).

Our aim was to identify gene(s) which regulate IL-4 and IL-10 expression in order to establish whether these Th2 cytokines are regulated independently or as a group. Background strain BALB/c produces high levels of IL-4 and IL-10, whereas donor strain STS is a low producer of these cytokines. In the pilot experiment we analyzed concanavalin A (ConA)-induced production of IL-4 and IL-10 in the CcS/Dem series (data not shown, M. Krulová, PhD thesis, Prague 1998). The strain CcS-20 exhibited the lowest expression of both IL-4 and IL-10 from all the CcS/Dem strains and was therefore selected for further analysis. In order to map the loci responsible for this difference we analyzed the F_2 hybrids between this RC strain and the parental strain BALB/c.

Materials and methods

Mice

Mice, both male and female, of strains BALB/cHeA (BALB/c), STS/A (STS), CcS-20 and F_2 hybrids between BALB/c and CcS-20, 10 to 26 weeks old, came from P. Demant's breeding colony. The F_2 hybrids were bred and shipped from Amsterdam to Prague in two separate groups with a three-month interval. The genetic composition of strain CcS-20 has been described in detail by Stassen and co-workers (1996). When used for these experiments, strain CcS-20 was in at least the 33rd generation of inbreeding and therefore highly homozygous.

Lymphocyte proliferation assay

Spleen cells (10^5 cells per well) were incubated in 96-well tissue culture plates in complete RPMI 1640 medium only or stimulated with 1.25, 2.5 or 5 μg/ml ConA as described elsewhere (Lipoldová et al. 1995). [^{3}H]-Thymidine (Institute for Research, Development and Utilization of Radioisotopes, Prague, Czech Republic) (0.5 μCi/well) was added to the cultures for the last 6 h of the 72 h incubation period.

Estimation of cytokine levels

The supernatants of tissue cultures described above were collected after 24, 48, or 72 h of incubation and assayed for cytokine presence. IL-4 and IL-10 were determined using the primary and secondary monoclonal antibodies (mAbs), respectively (11B11, BVD624G2; JES5-2A5, SXC-1) purchased from PharMingen (San Diego, Calif.). The ELISA was performed using the conditions recommended by PharMingen. The total IL-4 and IL-10 levels were estimated from the titration curves (as standards were used appropriate recombinant cytokines from PharMingen) using the curve fitter program KIM-E.

Genotyping of simple sequence length polymorphism by polymerase chain reaction

DNA was isolated from tails using a standard proteinase procedure (Laird et al. 1991). Strain CcS-20 carries genetic material of STS origin on 15 segments on 10 chromosomes. These segments were typed in F_2 hybrids using 24 microsatellite markers: *D1Mit14*, *D1Mit17* (chromosome 1); *D2Mit227*, *D2Mit74* (chromosome 2); *D3Mit25*, *D3Mit49*, *D3Mit11* (chromosome 3); *D4Mit149* (chromosome 4); *D5Mit112*, *D5Mit114*, *D5Mit20*, *D5Mit175*, *D5Mit26*, *D5Mit63*, *D5Mit143* (chromosome 5); *D6Mit23*, *D6Mit52* (chromosome 6); *D8Mit129* (chromosome 8); *D9Mit42*, *D9Mit2* (chromosome 9); *D14Mit20* (chromosome 14); *D16Mit15*, *D16Mit19*, *D16Mit7* (chromosome 16). The largest distance between any two markers derived from the STS strain or from the nearest BALB/c-derived marker was 18.2 cM. PCR genotyping was performed as described (Dietrich et al. 1992; Lipoldová et al. 1995), using the primers from Research Genetics (Huntsville, Fl.).

Statistical analysis

The statistical significance of the differences in cytokine production between strains as well as linkage of genetic factors controlling ConA-induced IL-4 and IL-10 expression were examined by analysis of variance (ANOVA, NCSS). Strain, genotype of F_2 hybrids, gender, and age were fixed factors, and individual experiments were considered a random parameter. In order to obtain normal distribution of the observed cytokine levels required for ANOVA, the measured values were transformed as described in Results or in legends to Figures and Tables. The differences between strains were evaluated by Newman-Keuls multiple comparison test at 95% significance. In linkage analysis, markers and interactions with $P < 0.05$ were analyzed in a single model. P values were corrected for multiple comparisons as described by Lander and Schork (1994). Pearson correlation coefficients were computed by the NCSS package. The significance of differences between the correlation coefficients in different genotypes was computed by dividing their difference by the square root of the sum of their standard deviations. The probability of this ratio has a z distribution.

Results

Differences in cytokine expression in BALB/c, STS, and CcS-20

We measured the levels of IL-4 and IL-10 in supernatants after ConA stimulation by 1.25, 2.5, and 5 μg/ml

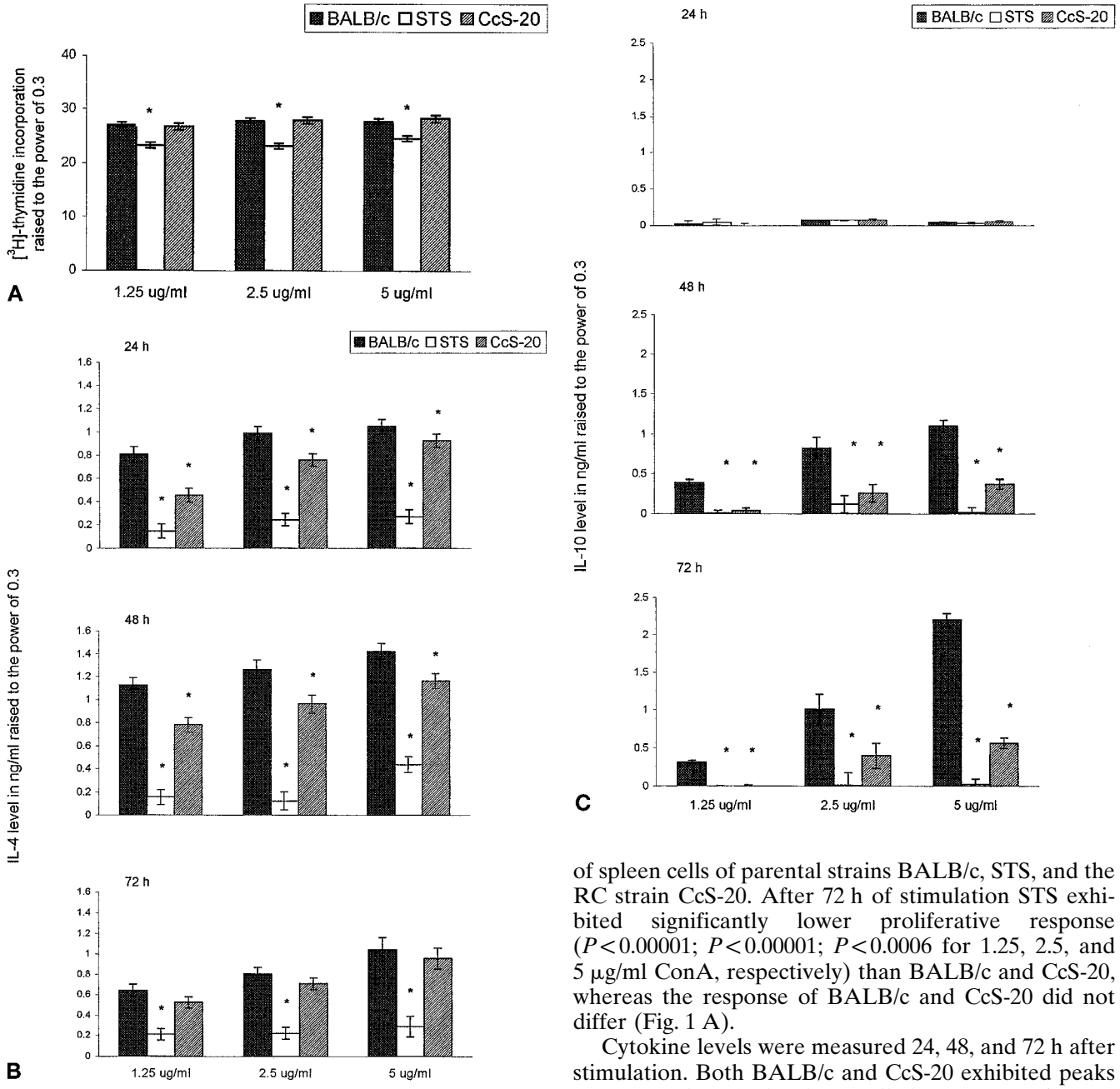

Fig. 1A–C Comparison of **A** the proliferative response, **B** IL-4, and **C** IL-10 expression of spleen cells stimulated by ConA. Spleen cells were stimulated by 1.25, 2.5, or 5 μg/ml ConA for 72 h and the proliferative response measured by [^{3}H]-thymidine incorporation. Cell supernatants were analyzed after 24, 48, and 72 h after ConA stimulation as described in Materials and methods. Data summarize the results of seven **A**, **B** and three **C** independent experiments. Both female and male mice were used in our analysis, but no influence of sex on strain differences was observed. The columns show the means of counts **A**, IL-4 **B** or IL-10 **C** levels in ng/ml raised to the power of 0.3. The means were computed by ANOVA including the sex and experiment as fixed and random factors, respectively. BALB ▩; STS □; CcS-20 ▨. *Asterisks* indicate significant difference from BALB/c: **A** 1.25 μg/ml $P<0.00001$; 2.5 μg/ml $P<0.00001$; 5 μg/ml $P<0.0006$ **B** $P<0.0005$; **C** $P<0.01$

of spleen cells of parental strains BALB/c, STS, and the RC strain CcS-20. After 72 h of stimulation STS exhibited significantly lower proliferative response ($P<0.00001$; $P<0.00001$; $P<0.0006$ for 1.25, 2.5, and 5 μg/ml ConA, respectively) than BALB/c and CcS-20, whereas the response of BALB/c and CcS-20 did not differ (Fig. 1 A).

Cytokine levels were measured 24, 48, and 72 h after stimulation. Both BALB/c and CcS-20 exhibited peaks of IL-4 and IL-10 levels for all the concentrations of ConA used at 48 and 72 h, respectively. CcS-20 expressed a lower level of IL-4 than BALB/c after 24 and 48 h of stimulation ($P<0.005$), whereas STS produced a very low level of this cytokine and differed from both BALB/c and CcS-20 ($P<0.00001$) (Fig. 1 B). Supernatants of spleen cells stimulated by ConA for 24 h contained very low levels of IL-10 and no differences were observed between individual strains; after 48 and 72 h of stimulation BALB/c produced higher titers of IL-10 than STS. CcS-20 differed from both parental strains and exhibited lower expression of IL-10 than BALB/c and higher than STS ($P<0.01$) (Fig. 1 C).

Table 1 Linkage analysis of IL-4 production of (CcS-20 × BALB)F_2 hybrids after ConA stimulation. The numbers give the level of IL-4 (in ng/ml) in spleen cell supernatants raised to the power of 0.4. The values of strains BALB/c, STS, and CcS-20 are shown in Fig. 1. S and C indicate the presence of the STS and BALB/c alleles, respectively. NS, not significant, is used for $P > 0.05$

Chromosome position (cM)	Marker	48 h										72 h				
		2.5 μg/ml					5.0 μg/ml					2.5 μg/ml				
		Genotype			Corrected		Genotype			Corrected		Genotype			Corrected	
		C/C	C/S	S/S	$P=$	$P=$	C/C	C/S	S/S	$P=$	$P=$	C/C	C/S	S/S	$P=$	$P=$
30.8	*D16Mit15*	1.96 ± 0.07 (n = 40)	1.98 ± 0.05 (n = 81)	2.25 ± 0.07 (n = 43)	0.0066	NS	2.08 ± 0.07 (n = 40)	2.18 ± 0.05 (n = 81)	2.53 ± 0.07 (n = 43)	0.00041	0.046	1.36 ± 0.07 (n = 39)	1.54 ± 0.05 (n = 73)	1.91 ± 0.07 (n = 43)	0.000041	0.0062
42.5	*D16Mit19*	1.98 ± 0.07 (n = 43)	1.98 ± 0.05 (n = 91)	2.11 ± 0.08 (n = 38)	NS	NS	2.29 ± 0.07 (n = 43)	2.19 ± 0.05 (n = 91)	2.48 ± 0.08 (n = 38)	0.0069	NS	1.54 × 0.07 (n = 44)	1.54 ± 0.05 (n = 81)	1.77 ± 0.08 (n = 34)	0.068	NS
50.4	*D16Mit7*	1.94 ± 0.07 (n = 44)	1.99 ± 0.05 (n = 90)	2.15 ± 0.08 (n = 38)	NS	NS	2.48 ± 0.07 (n = 44)	2.45 ± 0.05 (n = 90)	2.82 ± 0.07 (n = 38)	0.0016	NS	1.45 ± 0.07 (n = 42)	1.47 ± 0.05 (n = 82)	1.69 ± 0.08 (n = 36)	0.066	NS

Table 2 Linkage analysis of IL-10 production of (CcS-20 × BALB)F_2 hybrids after ConA stimulation. The numbers give the level of IL-10 (in ng/ml) in spleen cell supernatants raised to the power of 0.3 (the values in strains BALB/c, STS and CcS-20 are shown in Fig. 1). S and C indicate the presence of the STS and BALB/c alleles, respectively. NS, not significant, is used for $P > 0.05$

Chromosome position (cM)	Marker	72 h									
		2.5 μg/ml					5.0 μg/ml				
		Genotype			Corrected		Genotype			Corrected	
		C/C	C/S	S/S	$P=$	$P=$	C/C	C/S	S/S	$P=$	$P=$
84.9	*D1Mit14*	0.52 ± 0.01 (n = 13)	0.44 ± 0.01 (n = 36)	0.41 ± 0.01 (n = 16)	0.000065	0.011	0.49 ± 0.02 (n = 14)	0.41 ± 0.01 (n = 34)	0.38 ± 0.02 (n = 16)	0.0082	NS
84.9	*D1Mit227*	0.51 ± 0.01 (n = 13)	0.44 ± 0.01 (n = 39)	0.41 ± 0.01 (n = 16)	0.0001	0.016	0.47 ± 0.02 (n = 13)	0.40 ± 0.01 (n = 35)	0.35 ± 0.02 (n = 16)	0.0081	NS
114.4	*D1Mit17*	0.42 ± 0.01 (n = 13)	0.44 ± 0.01 (n = 39)	0.52 ± 0.01 (n = 14)	0.0025	NS	0.38 ± 0.02 (n = 12)	0.35 ± 0.01 (n = 38)	0.49 ± 0.02 (n = 14)	0.0052	NS
29.4	*D5Mit112*	0.45 ± 0.01 (n = 19)	0.43 ± 0.01 (n = 35)	0.42 ± 0.02 (n = 14)	NS		0.45 ± 0.02 (n = 19)	0.41 ± 0.01 (n = 35)	0.30 ± 0.02 (n = 13)	0.0019	NS
31.7	*D5Mit114*	0.44 ± 0.01 (n = 21)	0.40 ± 0.01 (n = 35)	0.40 ± 0.02 (n = 13)	NS		0.56 ± 0.02 (n = 19)	0.53 ± 0.02 (n = 34)	0.40 ± 0.02 (n = 15)	0.0009	NS
36.2	*D5Mit20*	0.46 ± 0.01 (n = 20)	0.45 ± 0.01 (n = 33)	0.41 ± 0.01 (n = 15)	NS		0.47 ± 0.02 (n = 19)	0.43 ± 0.02 (n = 33)	0.29 ± 0.02 (n = 15)	0.0003	0.039
41.8	*D5Mit175*	0.45 ± 0.01 (n = 21)	0.44 ± 0.01 (n = 32)	0.42 ± 0.01 (n = 16)	NS		0.53 ± 0.02 (n = 16)	0.53 ± 0.01 (n = 36)	0.44 ± 0.02 (n = 19)	0.0040	NS
47.4	*D5Mit26*	0.45 ± 0.01 (n = 20)	0.45 ± 0.01 (n = 32)	0.44 ± 0.02 (n = 14)	NS		0.35 ± 0.02 (n = 19)	0.33 ± 0.02 (n = 32)	0.27 ± 0.02 (n = 14)	NS	
64.3	*D5Mit29*	0.43 ± 0.01 (n = 19)	0.44 ± 0.01 (n = 35)	0.41 ± 0.01 (n = 16)	NS		0.45 ± 0.02 (n = 18)	0.43 ± 0.02 (n = 35)	0.34 ± 0.02 (n = 16)	0.0033	NS
65.4	*D5Mit63*	0.45 ± 0.01 (n = 21)	0.47 ± 0.01 (n = 38)	0.43 ± 0.02 (n = 17)	NS		0.44 ± 0.02 (n = 20)	0.43 ± 0.01 (n = 38)	0.32 ± 0.02 (n = 17)	0.0007	0.080

Linkage analysis of IL-4 and IL-10 production in F_2 hybrids between BALB/c and CcS-20

We examined the IL-4 expression in 164 F_2 hybrids after stimulation with 2.5 and 5 μg/ml ConA. Concentration of IL-4 was raised to the power of 0.4 to obtain normal distribution.

Statistical evaluation revealed linkage of the IL-4 expression induced by 5 μg/ml ConA for 48 h and 2.5 μg/ml ConA for 72 h to the marker *D16Mit15* on chromosome 16; corrected $P=0.046$ and 0.0062, respectively (Table 1). The STS allele determines higher IL-4 concentration in cell supernatants (Table 1). No strong evidence of a genotype and gender interaction was found ($P=0.91$ and 0.049 for group 1 and group 2, respectively). Influence of experiment was observed $P<0.00001$. These data indicate the presence of a locus *Cypr1* (cytokine production 1) in this region, controlling the IL-4 level induced by polyclonal stimulation by ConA.

Expression of IL-10 was tested in 80 F_2 hybrids. Spleen cells were stimulated by 2.5 or 5 μg/ml ConA for 72 h. Concentration of IL-10 in cell supernatants was raised to the power of 0.3 to obtain normal distribution. We found linkage of IL-10 expression after stimulation by 2.5 μg/ml ConA to the markers *D1Mit14* and *D1Mit227* (corr. $P=0.0111$ and 0.0155, respectively), locating the responsible locus, *Cypr2,* in the distal end of chromosome 1. The higher production was seen with the BALB/c allele of this locus (Table 2). When the concentration of 5 μg/ml ConA was used, IL-10 level was controlled by another locus, *Cypr3,* near the *D5Mit20* marker in the central part of chromosome 5. The BALB/c allele determines higher production of IL-10. No interaction between genotype and gender was observed.

Genotype influences correlation between IL-4 and IL-10 net production

We compared the expression of IL-4 and IL-10 by spleen cells of 80 F_2 hybrids stimulated by ConA for 72 h, and we observed a weak correlation between expression of these two Th2 cytokines when the splenocytes were stimulated by 5 μg/ml ConA ($R=0.378$, $P<0.0006$) and no correlation when the concentration 2.5 μg/ml was used ($R=0.056$, $P=0.621$). As this relationship might reflect different kinetics in expression of these interleukins, we also analyzed the correlation between production of IL-4 after 48 h and IL-10 after 72 h. The correlation was even lower: $R=-0.178$, $P=0.114$ and $R=0.300$, $P=0.007$ for 2.5 μg/ml and 5 μg/ml respectively.

We then determined in F_2 hybrids how different loci influence the correlation between expression of IL-4 and IL-10. We observed the influence of genotype at a novel locus *cora1* (correlation 1) marked by *D5Mit63* on chromosome 5 (Table 3) on the correlation between production of IL-4 and IL-10 after stimulation with

Table 3 Genetic influence on correlation between IL-4 and IL-10. IL-4 and IL-10 levels in ConA-stimulated cell supernatants from (CcS-20×BALB)F_2 hybrids (n=80) estimated by ELISA and expressed in ng/ml. Correlation between IL-10 measured after 72 h and IL-4 expression after 48 and 72 h was estimated for stimulation of both 2.5 μg/ml and 5 μg/ml ConA

Marker	2.5 μg/ml							5 μg/ml								
	IL-4 48 h			IL-4 72 h				IL-4 48 h			IL-4 72 h					
	Genotype		$Z=$	$P=$	Genotype		$Z=$	$P=$	Genotype		$Z=$	$P=$	Genotype		$Z=$	$P=$	Total $Z=$	Total $P=$	Corrected $P=$
D5Mit29	−0.02 (n=20)	−0.15 (n=36)	0.33 (n=16)	1.59	0.0559	0.16 (n=20)	0.43 (n=36)	0.42 (n=16)	1.46	NS	0.14 (n=19)	−0.08 (n=36)	0.31 (n=16)	1.30	NS	0.49 (n=19)	0.08 (n=36)	0.23 (n=16)	0.50	NS	2.43	0.0078	NS
D5Mit63	−0.06 (n=23)	−0.18 (n=39)	0.42 (n=17)	2.08	0.0188	0.14 (n=23)	0.10 (n=39)	0.47 (n=17)	1.25	NS	0.15 (n=22)	−0.07 (n=39)	0.31 (n=17)	1.30	NS	0.57 (n=22)	0.08 (n=39)	0.32 (n=17)	0.85	NS	2.74	0.0032	0.0448

ConA. Higher correlation was observed in homozygotes in either *C* or *S* allele than in heterozygotes (corr. $P = 0.0448$).

Discussion

We observed that IL-4 and IL-10 expression is regulated independently by loci on chromosome 16 and chromosomes 1 and 5, respectively. In addition, correlation between IL-4 and IL-10 is influenced by a locus near *D5Mit63* on chromosome 5 (Fig. 2). The original Th1/Th2 paradigm proposes two main groups of $CD4^+$ T-helper (Th) cells that have distinct patterns of cytokine production (Mosmann et al. 1986). Th1 cells secrete IL-2, TNFα, and IFNγ, whereas Th2 cells produce IL-4, IL-5, IL-10, and IL-13. Th1 and Th2 cells develop from Th0 cells, which usually arise in the absence of polarizing signals and express IL-2, IL-4, TNF, and IFNγ. It has been proposed that a locus *Tms1* on the central part of chromosome 11 controls Th1/Th2 dichotomy under neutral condition in vitro (Gorham et al. 1996). However, there is an increasing number of data indicating that the Th1/Th2 paradigm may be an oversimplification (Allen and Maizels 1997) and that cytokine-producing T cells cannot be classified into discrete subsets (Kelso 1995). Heterogeneity of single-cell cytokine gene expression in a clonal T-cell population was observed and the frequency of IL-4-expressing cells was higher than that of IL-5- and IL-10-expressing cells in the same Th2 population (Bucy et al. 1994). In our experiments, using polyclonal stimulation of spleen cells in the absence of polarizing signals we show that the two Th2 cytokines are at this stage of T cell development regulated independently and not as a group, and we identified three loci which influence their expression.

Cypr1 marked by *D16Mit15* is situated on centromeric part of this chromosome in the 3.4 cM-long STS-derived segment encompassing no genes with a known immune function. In the near vicinity of this region is *Aod1,* the immunoregulatory locus controlling abrogation of tolerance in neonatal thymectomy-induced autoimmune dysgenesis (Wardell et al. 1995). It has been shown that thymectomized animals exhibited an increase in the frequency of mitogen-induced $CD4^+$ IL-4 producers (Bonomo et al. 1995) and therefore *Cypr1* might be involved in the regulation of this process. The relative positions of loci *Aod1* and *Cypr1* must be es-

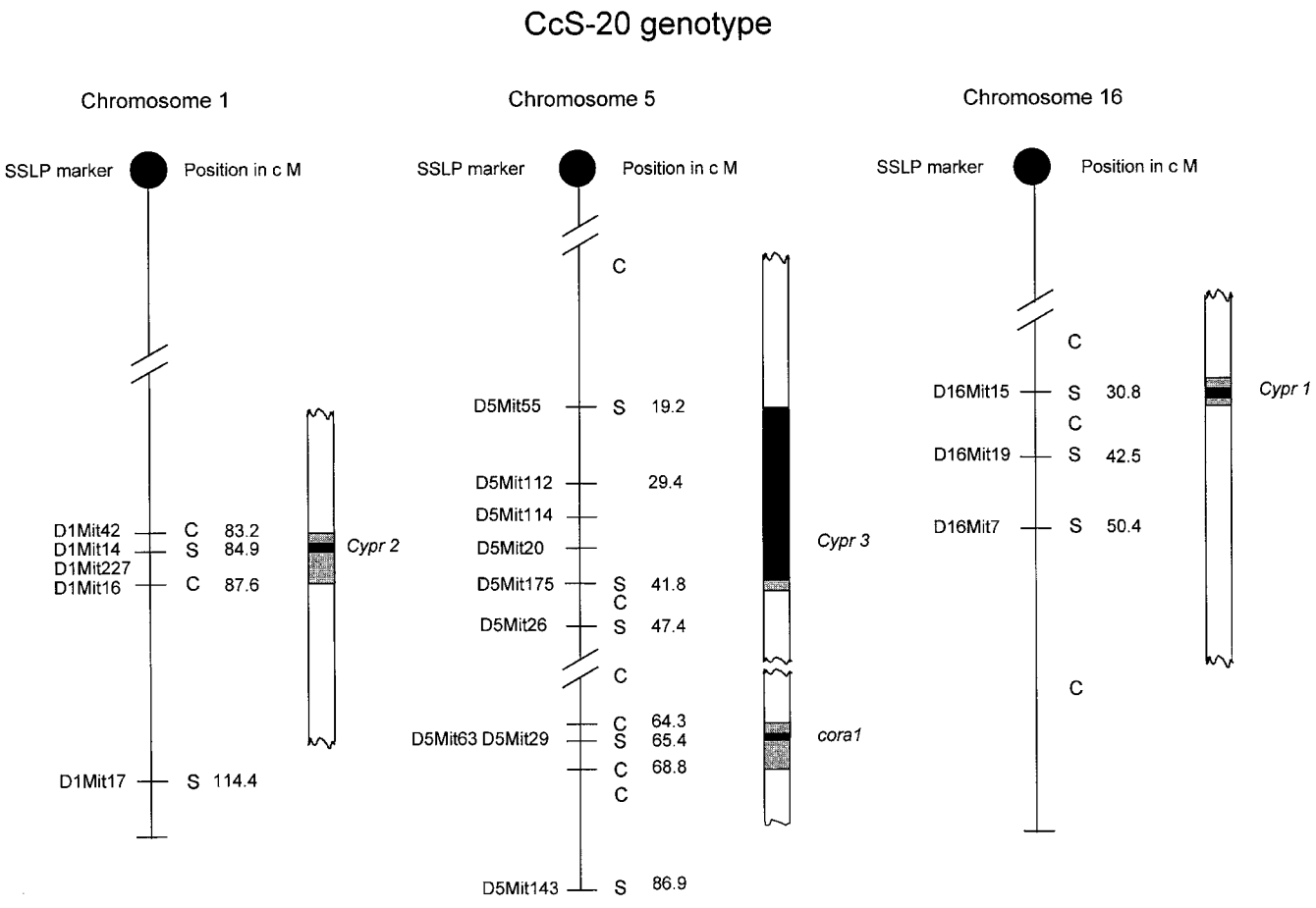

Fig. 2 Localization of *Cypr2*, *Cypr3*, *cora1*, and *Cypr1* on chromosomes 1, 5, and 16, respectively. The dark regions indicate the most likely location of these loci. The shaded regions indicate the maximal possible extent of the donor strain segments of chromosomes 1, 5, and 16 in CcS-20. C, BALB/c allele; S, STS allele

tablished by recombinant mapping (e. g., Moen et al. 1996).

Strain CcS-20 is an intermediate producer of IL-4, whereas BALB/c mice express a high level of this cytokine after polyclonal stimulation with ConA. However, the STS allele of *Cypr1* present in CcS-20 is associated with a high production of IL-4. This finding is not unique: susceptibility alleles originating from resistant strains were found in studies of liver and colon tumor susceptibility (Lee et al. 1995; van Wezel et al. 1996) and a low responder allele was identified in a strain exhibiting high response to IL-2 (Krulová et al. 1997). We did not detect any of the STS low-IL-4-producer alleles. These loci might possibly escape mapping if they are located on a very short segment of STS genome that was not detected during the typing of CcS/Dem strains. Alternatively, the lower production of IL-4 by the strain CcS-20 may be caused by the interaction of several loci which were not uncovered in our tests.

Cypr2 marked by *D1Mit14* is localized on the distal STS-derived segment on chromosome 1 which is 9.2 cM long. In this segment is localized also *Cd3z* (CD3 antigen, zeta polypeptide), which participates in T-cell activation (Exley et al. 1994); however, no influence of a polymorphism of this gene on IL-10 expression has been described. Three segments of STS origin are present on chromosome 5. *Cypr3* is situated in the central 28 cM-long segment, a locus *cora1* marked by *D5Mit63* influencing correlation between IL-4 and IL-10 expression is on the distal part (Fig. 2) in regions with no obvious candidate genes (Mouse Genome Database http://www.informatics.jax.org/mgd.html). Whereas the effect of the *Cypr2* gene were seen in experiments with a lower concentration of ConA (2.5 μg/ml), at a higher concentration (5 μg/ml) of ConA the level was controlled by *Cypr3*. These variations might be caused by an induction of different signaling pathways depending on ConA concentration. We have observed a similar phenomenon when studying proliferation induced by IL-2. The response to different concentrations of IL-2 is controlled by different loci, *Cinda1* and *Cinda2* (Krulová et al. 1997).

In the present study we found that different loci control expression of IL-4 and IL-10, and we show that the relationship between the level of these two Th2 cytokines depends on the genotype. This differential genetic regulation may be relevant for understanding biological effects of T-helper cells in mice of different genotypes.

Acknowledgments The authors would like to thank Mr. Joost de Moes for expert technical assistance. This work was supported by grant A5052604 from the Grant Agency of the Czech Academy of Sciences and grant 310/96/1311 from the Grant Agency of the Czech Republic to M. L., and the grants of the European Commission nr. SCO1*CT890213 and CHRXCT930181 to P. D.

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