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CCL23 Expression Is Induced by IL-4 in a STAT6-Dependent Fashion

Hermann Novak, Anke Müller, Nathalie Harrer, Claudia Günther, Jose M. Carballido, and Maximilian Woisetschläger

The chemokine CCL23 is primarily expressed in cells of the myeloid lineage but little information about its regulation is available. In this study, it is demonstrated that IL-4 and IL-13 induced CCL23 expression in human peripheral blood monocytes. GM-CSF had no effect on its own but synergized with IL-4, but not IL-13. CCL23 promoter reporter gene constructs were sensitive to IL-4 stimulation in the presence of the transcription factor STAT6. A canonical STAT6 binding site in the promoter region of the CCL23 gene was critical for the IL-4-inducible phenotype because reporter plasmids with a defective STAT6 binding site were unable to respond to IL-4 stimulation. In addition, two tandem copies of the STAT6 site conferred cytokine responsiveness to a heterologous minimal promoter. Furthermore, IL-4 inducibility of the CCL23 promoter was dependent on the absence of a negatively acting cis-element downstream of the STAT6 binding site. The negative function of this element was operative also on heterologous IL-4-inducible promoters. CCL23 was also expressed in skin from patients suffering from atopic dermatitis at higher levels than in normal individuals. However, no correlation between CCL23 expression in the serum and IgE levels as a diagnostic marker for atopy was found. Collectively, these data suggest a link between the inducible phenotype of CCL23 expression in monocytes by the prototype Th2 molecule pair IL-4/STAT6 and the increased number of CCL23-expressing cells in skin of atopic dermatitis patients.


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2 Abbreviations used in this paper: DC, dendritic cell; Ct, cycle threshold; EF1-α, elongation factor 1α.

Materials and Methods

Cell culture and cytokines

Human primary monocytes were isolated by counterflow centrifugal elutriation (29) from PBMC derived from healthy volunteer donors. The

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Chemokines are small proteins which regulate cell trafficking during homeostasis as well as during inflammatory processes. Chemokine subfamilies have been defined by the distance between the first two conserved cysteine residues with the CXC and the CC subfamily containing the vast majority of all chemokines. CXC chemokines act mainly on neutrophils and activated T lymphocytes (1), while CC chemokines attract a wider number of cell types including monocytes (2, 3), lymphocytes (4–6), basophils (7, 8), and eosinophils (9, 10).

CCL23 (MPIF-1, CKJ8, SCYA23), a member of the CC chemokine family, was originally isolated from a human aortic endothelial cell library (11) and from the human monocytic cell line THP-1 (12). It is most closely related to receptors and neutrophils (11), osteoclast precursor cells (17), and endothelial cells (18). In contrast, CCL23 reduces the proliferation of progenitor cells giving rise to granulocyte and monocyte lineages (11), whereas it enhances angiogenesis of endothelial cells (18). Little is known about the regulation of CCL23 expression. In monocytes, IL-1β and IFN-γ, albeit at low levels, induce CCL23 expression (16). Maturation of monocyte-derived DC with agonistic CD40 Abs or IFN-γ reduces CCL23 RNA and protein expression. In contrast, treatment of immature DC with IL-10 induces CCL23 production (15).

A major constituent of the IL-4 signal transduction pathway is STAT6 (19, 20). This protein is a transcription factor that resides in the cytoplasm of quiescent cells in a latent state. Upon binding of IL-4 to its receptor, the α-chain of the IL-4R becomes phosphorylated by the tyrosine kinases JAK1 and JAK3. The phosphorylated tyrosine residues on the IL-4R serve as docking sites for STAT6 molecules. Once recruited to the receptor, STAT6 also becomes phosphorylated by JAKs at a single tyrosine residue. Activated STAT6 dissociates from the receptor, dimerizes, and translocates into the nucleus, where it binds to specific sequences found within promoters of IL-4-regulated genes. STAT6 binding sites have been found in the promoter regions of various IL-4-inducible genes such as FIZZ1 (21), CD23 (22), eotaxin-1 (23, 24), eotaxin-3 (25), IL-4R (26), and in Ig germ line e (27) and γ1 promoters. The consensus binding sequence of STAT6 consists of the palindromic TCC sequence separated by four nucleotides 5’TTC(N)4GAA-3′ (28).

This study demonstrates that CCL23 production is specifically induced by IL-4 and IL-13 on human monocytes. The activating function of IL-4 is mediated by the STAT6 protein and is dependent onthe presence of a STAT6 binding site in the promoter region of the CCL23 gene. CCL23-expressing cells are found at higher frequency in the epidermis of patients suffering from atopic dermatitis compared with normal individuals. The chemokine was also detectable in plasma of normal and atopic individuals but no correlation with IgE as marker of atopy was found.
purity of these preparations assessed by CD14 staining was typically >96%. Cells were cultured at a density of 1 × 10^6/ml in 96-well plates at 37°C with 5% CO₂ in IMDM supplemented with 2% heat-inactivated FCS (Invitrogen Life Technologies), 100 U/ml penicillin, and 100 μg/ml streptomycin.

HEK293 cells were cultured at 37°C with 5% CO₂ in DMEM supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Purified human rIL-4 and GM-CSF were obtained from R&D Systems and were used at a concentration of 40 and 50 ng/ml, respectively. Commercially available IL-13 (PeproTech) was used at 50 ng/ml. LPS (Sigma-Aldrich) was used at 500 ng/ml.

**ELISA for CCL23**

Human CCL23 protein levels were measured using a standard sandwich ELISA. Microtiter wells (Maxisorp Immuno Plates; Nunc) were coated with 0.5 μg/ml anti-human CCL23 Ab MAB571 (R&D Systems) overnight at +4°C. Samples were added in different dilutions for 90 min at 37°C. As standard, recombinant CCL23, 22–120 was used (R&D Systems). Afterward, the immune complexes were detected by incubation with 0.5 μg/ml biotinylated CCL23 Ab (R&D Systems) for 90 min at 37°C. The complexes were made visible by adding streptavidin-linked HRP. The lower limit of sensitivity of the assay was 300 pg/ml.

**IgE determination**

Total IgE was determined in human plasma samples using an ImmunoCAP Total IgE kit (Pharmacia) on the Immuno-CAP-250 device (Pharmacia) as recommended by the supplier.

**Immunohistology**

Human skin biopsies (4-mm punch biopsies) were obtained from healthy volunteers and from patients with atopic dermatitis after informed consent. Tissue samples were embedded in OCT medium (Tissue-Tek) and kept at −80°C. Cryostat sections of 5 μm were fixed with dried acetone (Merck 1.00299) and stained with 10 μg/ml mouse IgG1 anti-human CCL23 mAb MPF-1 (clone 69512.111; R&D Systems) or with control mouse serum. Expression of CCL23 was detected by using the DakoCytoVision EnVision+ System-HRP according to manufacturer’s instructions. Samples were subsequently counterstained with hematoxylin (Vector Laboratories), mounted using Faramount aqueous mounting medium (DakoCytomation), and analyzed in an Axiosplana2 microscope (Zeiss) equipped with a digital Spot camera (Visitron Systems).

**Total RNA isolation, cDNA synthesis, and RT-PCR**

Total RNA was isolated using the Absolutely RNA Miniprep kit (Stratagene) according to the instructions of the manufacturer. cDNA was synthesized from 0.7 μg of RNA by reverse transcription using iScript reverse transcriptase (Bio-Rad) in a total volume of 20 μl according to the instructions of the manufacturer.

The expression of CCL23 was analyzed with the commercially available SYBR Green RT-PCR kit (Applied Biosystems.) in an ABI7900 machine with the following PCR parameters: one cycle of 2 min at 50°C and 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 62°C. All templates were analyzed in duplicates and the average was used for further calculation. The average cycle threshold (CT) values of the samples were normalized to the average Ct values of the housekeeping gene elongation factor 1-α (EF1-α) using the following equation: 2^{ΔΔCT} \times 10^{100}. The following primer pairs were used: CCL23, 5'-ATGCTGTGTA CTCGCCCTTTG-3' and 5'-GGCGCTCTGGAGGACATC-3' and EF1-α, 5'-TTTTGAGGACCAAGATCAGTGA C-3' and 5'-TACGCGCTTGA GGTCCCTGTAA-3'.

**Recombinant plasmids**

Various DNA fragments containing the human CCL23 promoter were amplified from human genomic DNA using the upstream PCR primer 5'-CTGAGACTGAGGATCCTATTTACTGCTCAGTTCGC-3' along with the reverse primers 5'-AGTCAAGTGTCATCTGCGTCATCTGCTGTC-3' (CCL23p-XH), 5'-AGTCAAGTGATCTATAGCTTCTGCTCAGTTCGTC-3' (CCL23p-XE plus XH), 5'-AGTCAAGTGATCCTATTTACTGCTCAGTTCGC-3' (CCL23p-XE plus XH), and 5'-AGTCAAGTGATCCTATTTACTGCTCAGTTCGC-3' (CCL23p-XE plus XE), a 558-bp XhoI/XbaI fragment (CCL23p-XX), and a 881-bp XhoI/AccI fragment (CCL23p-XA) were ligated into the same vector backbone. Site-directed mutations in the STAT6 site in construct CCL23p-XEmut were introduced by two sequential PCR as described previously (31) using the mutant primers 5'-GAGAATAATGGGGAGTTT TATTTGGAAGAATAAGAG-3' and 5'-ATCTTATTTCTCTTCCA AAAAATAACTCCCTTCTCTTCTTCAGA-3'. Plasmid STAT6mBG was created by ligating a double-stranded oligonucleotide with XhoI/BglII overhangs into the constructs EOT3 (25) and mBGL-NUC nf-κB1 (30) upstream of the STAT6 sites using the following sequences: 5’-CCCTTCTGAGACTAAGCGGCAGG CTGCTAGAGAAGGAA-3’ and 5’-GATCTTCTTCTCCTAAAATAA AATCCTCC-3’.

Transient transfection of HEK293 cells

Transient transfection of HEK293 cells was achieved using calcium phosphate coprecipitation (24). Briefly, the transfectants were washed 24 h after transfection and cultured in fresh medium in the presence or absence of 40 ng/ml IL-4 for another 24 h before luciferase assays were conducted in duplicates according to the instructions of the manufacturer using the Promega Luciferase Assay System (Promega).

**Preparation of nuclear extracts and EMSA**

Nuclear extracts from unstimulated human primary blood monocytes or cells that had been stimulated for 4 h with IL-4, GM-CSF, or both were prepared according to the method described by Andrews and Faller (32). A double-stranded oligonucleotide probe containing the STAT6 binding site in the CCL23 promoter was end-labeled using [α-32P]dCTP (Amersham Biosciences) and Klenow polymerase (Roche Diagnostics). The nuclear-protein-binding reaction was done as described previously (33) using 5 μg of nuclear extracts. For oligonucleotide competition experiments, extracts were competed with various amounts of unlabeled competitor oligonucleotide on ice for 30 min before the radiolabeled probe was added. The following oligonucleotides were used in these experiments: CCL23, 5'-TCGAGAGGTAAAAATTGGGAGTTTTTGTATTTTAGAAGGAAATGGGAGTAG TATTTTGTGTTGAAA-3‘ and 5’-CCGGCTCTTCTCTCAGCCTGTTA GCTTCGAGGAAAGGTTAC-3’.

**Results**

DC have been shown to express CCL23 (15). Because these cells can be generated from monocytes by IL-4 and GM-CSF, it was determined whether CCL23 production was regulated in monocytes during the differentiation process into DC. Monocytes were incubated with IL-4, GM-CSF, or the combination of the two cytokines and the expression of CCL23 gene transcripts was measured by quantitative RT-PCR. The constitutive production of CCL23 mRNA was almost undetectable in unstimulated cells. IL-4, but not GM-CSF induced the synthesis of significant amounts of CCL23 transcripts within 24 h. The combination of the cytokines had a synergistic effect in all 10 donors analyzed (Fig. 1A). The mean factor of induction at 24 h with IL-4/GM-CSF was 375 ± 91-fold depending on the donor. Because IL-13 is known to share most of the biological activities with IL-4, monocytes were stimulated with IL-13 alone or in combination with GM-CSF for 3 days to assess whether this cytokine was also able to promote CCL23 expression. The results demonstrated that, like IL-4, IL-13 alone was sufficient to induce CCL23 expression. The response to IL-13 was always significantly lower than after IL-4 induction. However, in contrast to IL-4, GM-CSF had no synergistic effects (Fig. 1B). When monocytes were stimulated with LPS plus IFN-γ, no activation of CCL23 expression was observed (data not shown).

A kinetic study showed that maximal CCL23 expression was reached at day 3 in IL-4 plus GM-CSF-stimulated monocytes (Fig.
CCL23 induction in monocytes. A representative experiment of 10 is shown. A, CCL23 steady-state mRNA expression in monocytes is depicted relative to the housekeeping gene EF1-α. B, GM-CSF synergizes with IL-4 but not with IL-13 to stimulate CCL23 expression in monocytes. Error bars, SDs. C, Time course of IL-4- and GM-CSF-induced CCL23 RNA and protein expression by monocytes. Error bars, SDs.

Figure 2. A, The schematic structure of four different CCL23 promoter reporter gene constructs is shown. The numbers refer to the number of nucleotides relative to the start site of transcription (bent arrow). TATA indicates the minimal promoter from the mouse β-globin gene linked to the firefly luciferase reporter (Luc). The black box marks the position of the STAT6 site. The nucleotide sequence of the STAT6 site in the CCL23 promoter is shown along with known STAT6 sites from the eotaxin-3 and the IgE germline promoter. Shaded nucleotides highlight the two arrows indicate the position of the inverted repeat triplet in the STAT6 site. B, IL-4 inducibility of CCL23 reporter plasmids in transiently transfected HEK293 cells. The induction factor represents the ratio of luciferase expression in IL-4-treated vs uninduced cells. One representative experiment of four is shown.
transduction pathway are intact. Therefore, HEK293 cells are a suitable tool to study the involvement of STAT6 in IL-4-driven cellular responses (35). The transfectants were stimulated with IL-4 and the response of the reporter plasmids to the cytokine was monitored by measuring the enzymatic activity of luciferase. The results demonstrated that only plasmid XE harboring a short XhoI/EcoRV promoter fragment was inducible with IL-4 while the other three reporter constructs (XH, XA, and XX) with longer promoter sequences at the 3’ end were not responsive (Fig. 2B). The sensitivity of the XE plasmid toward IL-4 was dependent on the presence of cotransfected STAT6 (Fig. 2B, right panel). These data showed that the 421-bp promoter segment in XE contained cis-acting sequences mediating IL-4 responsiveness. In addition, sequences between the EcoRV and the XhoI site must contain cis-acting elements which acted in a dominant negative fashion by suppressing the cytokine response.

To explore whether the putative STAT6 site was functionally involved in IL-4-mediated activation of the XE construct, two point mutations were introduced into the 5’-located direct repeat TTC (Fig. 3, upper panel). Such mutations have been shown earlier to abrogate the function of bona fide STAT6 binding sites (25). Transient transfection of the mutant reporter plasmid XEmut along with the STAT6 expression vector in HEK293 cells revealed that IL-4 was unable to induce promoter activation, demonstrating that this site was critically involved in the cytokine response (Fig. 3, lower left panel). To further prove the function of this site as IL-4 response element in the absence of any other CCL23 promoter sequences, two tandem wild-type or mutated copies were cloned upstream of a heterologous TATA box in the vector mBG-luc (30). Transfectants carrying plasmid STAT6mBG responded to IL-4 with an 8-fold increase in luciferase expression while cells transfected with STAT6mut-mBG did not respond to cytokine stimulation (Fig. 3, lower right panel). Again, IL-4 stimulation of STAT6mBG was dependent on the presence of cotransfected STAT6 (data not shown).

FIGURE 3. The CCL23 STAT6 site is required for IL-4 induction of the CCL23 promoter. Top, The schematic structure of the CCL23p-XE reporter plasmid is shown. The black boxes denote the position of the STAT6 site. Below, the sequence of the wild-type (CCL23p-XE) and mutated STAT6 site (CCL23p-XEmut) is indicated. Left graph, The IL-4 response of the two CCL23 promoter plasmids. Right graph, The cytokine inducibility of the STAT6 site in the absence of additional CCL23 promoter elements. One representative of six experiments is shown.

To directly demonstrate binding of STAT6 to the 5’TTCCTTTGTCAAA-3’ element, EMSA experiments were conducted. Incubation of a radiolabeled double-stranded oligonucleotide containing the putative STAT6 binding site with nuclear extracts derived from unstimulated primary monocytes or cells stimulated with IL-4, GM-CSF, or IL-4 plus GM-CSF revealed the formation of two distinct bands detectable with all four extracts. In extracts from IL-4- and IL-4 plus GM-CSF-treated monocytes, a third more slowly migrating nucleoprotein complex was observed (Fig. 4, left panel). This band was specifically induced by IL-4 because it was absent in extracts derived from unstimulated or GM-CSF-treated cells. Moreover, its migratory behavior was identical to the STAT6-containing complex formed when the same extracts were incubated with the functional STAT6 binding site identified in the eotaxin-3 promoter (25) (Fig. 4, right panel). These data strongly indicated that STAT6 interacted with the IL-4-responsive element. Competition experiments were used to compare the relative strength of the interaction of STAT6 to the CCL23 vs the eotaxin-3 binding site. Titration of different amounts of unlabeled CCL23 or eotaxin-3 STAT6 binding site oligonucleotides showed that a 3-fold molar excess of the eotaxin-3 STAT6 site double-stranded oligonucleotide efficiently competed for binding to the labeled eotaxin-3 STAT6 probe (Fig. 4, right panel). In contrast, a 10-fold higher concentration was needed to achieve a similar degree of competition with the CCL23 STAT6 sequence. These data suggested that the CCL23 STAT6 motif bound STAT6 with low affinity compared with the eotaxin-3 recognition site.

As shown in Fig. 2, a dominant negative acting regulatory element was located between the EcoRV and the XhoI site in the CCL23 promoter. To map this element in more detail, additional reporter constructs were made with progressive extensions at the 3’ end (Fig. 5, upper panel). IL-4 inducibility of these plasmids was analyzed in transiently transfected HEK293 cells in the presence of the STAT6 expression vector. Construct XE + 30 led to a small but statistically significant decrease in cytokine responsiveness compared with construct XE, suggesting that this stretch of DNA contributed to the negative regulatory function. In contrast, IL-4 could not induce luciferase expression in cells transfected with the plasmids XE + 60 and XE + 90 (Fig. 5, lower left panel), demonstrating that the 30 additional nucleotides in XE + 60 vs XE + 30 (top of Fig. 5) were critically involved in repression of the IL-4 response. To explore whether the negative function of this DNA stretch was specific for the CCL23 gene, a single copy of this sequence was cloned into two reporter plasmids that had been shown earlier to be inducible with IL-4 in a STAT6-dependent
fashion. Plasmid EOT3 contains the human eotaxin-3 promoter (25), whereas plasmid (IL4RE)2Luc contains two copies of the STAT6 site identified in the human IgE germline gene promoter (30). In both cases, the negative CCL23 promoter element was placed upstream of the STAT6 site. As shown in Fig. 6, lower right panel, the cytokine response of EOT3–30/60 and (IL4RE)2Luc-30/60 was significantly reduced compared with the parental plasmids. It should be noted, however, that the repressing effect was only partial and not complete as observed with the CCL23 XE plasmid. These data demonstrated that the negative function of this 30-nt DNA element was not limited to the CCL23 gene but could be transferred to other IL-4/STAT6-driven genes.

The IL-4/STAT6 molecule pair is well known in Th2 cell-mediated immune reactions and is critically involved in atopic diseases like atopic dermatitis or allergic asthma (36). Therefore, the possibility that CCL23 expression may be associated with atopy was investigated. To determine whether CCL23 was expressed in blood and whether differences in expression existed between healthy and atopic individuals, plasma from 57 individuals was analyzed for CCL23 protein by ELISA. Fifteen samples were positive for CCL23 (26% of all samples) with concentrations varying over a wide range between 8.7 ng and 300 pg, which was the lower limit of detection (Table I). Based on the moderate sensitivity of the ELISA, it is quite possible that the percentage of donors containing biologically relevant concentrations of CCL23 is much higher than 26%. Serum IgE levels higher than 100 kU/L serve as one of several parameters indicative of atopy in adults (37). Thus, the same plasma samples were analyzed for IgE concentrations (Table I). Thirteen of 57 donors had IgE plasma levels higher than 100 kU/L. Among those, three samples had measurable CCL23 bioreactivity (23%). Among the 44 individuals with IgE concentrations <100 kU/L, 12 were positive for CCL23 (27%). Thus, the percentage of CCL23-positive samples was very similar in both the high- and low-IgE groups, indicating no correlation between serum CCL23 and elevated IgE levels.

To determine whether CCL23 is expressed in skin and whether differences could be detected in tissue derived from patients suffering from atopic dermatitis, skin samples were assayed for the presence of CCL23-producing cells by immunohistochemistry. CCL23 immunoreactivity was not or only very sparsely observed in the skin of normal human subjects (Fig. 6C). In contrast, CCL23-immunoreactive cells appeared more frequently in the skin of atopic dermatitis patients (Fig. 6B).
Discussion

This study extends the distribution of expression of CCL23 to human skin and blood. A sizeable proportion of the individuals tested expressed the chemokine in these two organs. So far, CCL23 transcripts have been observed in pancreas, skeletal muscle (12), lung, and liver (11). The expression of CCL23 in blood is remarkable because only a small subset of chemokines can be readily detected in plasma such as CCL14 and CCL15 (38).

Cultivation of blood monocytes with IL-4 plus GM-CSF resulted in activation of CCL23 expression both on the RNA and the protein level. CCL23 production was maximal at 3 days. This time course is reminiscent of the one required for IL-4/GM-CSF-driven transcription. CCL23 transcripts have been observed in pancreas, skeletal muscle (12), lung, and liver (11). The expression of CCL23 in blood is remarkable because only a small subset of chemokines can be readily detected in plasma such as CCL14 and CCL15 (38).

The sustained expression of CCL23 is in contrast to the transient induction of chemokine expression upon IL-1β treatment which peaks at 8 h and is rapidly lost thereafter (16). Although GM-CSF had no inducing effect on its own, it synergized with IL-4. A number of possible explanations may account for the mechanistic basis of the synergistic effect. GM-CSF receptor engagement could induce synergy at the transcriptional level by activating a costimulatory transcription factor which cooperates with STAT6. Such a mechanism has been described earlier for the IgE germ line gene which requires the cooperation between STAT6 and other factors such as NF-κB and PU.1 for full gene activation by IL-4 plus CD40 signaling (30, 34). It is also possible that GM-CSF signaling leads to the derepression or shutdown of a negative-acting factor. Alternatively, GM-CSF may act at the posttranscriptional level by stabilizing CCL23 mRNA. This possibility appears less likely because no synergistic effect of GM-CSF was found with IL-13.

IL-4 inducibility of the CCL23 gene was dependent on a STAT6 binding site in the regulatory region of the gene. This claim is primarily based on the results in the HEK293 cell system. Indirect support that STAT6 is also involved in regulating CCL23 expression in primary monocytes comes from the fast kinetics of CCL23 induction in monocytes where mRNA was clearly detectable already at 4 h upon IL-4/GM-CSF treatment (data not shown). This fast kinetics is typically found for a number of STAT6-regulated genes (21, 24, 25). The STAT6 site is located between −698 and −689 relative to the transcriptional start site. This is remarkable because the majority of STAT6 sites are found in the proximal promoter region within 250 bp relative to the transcriptional start site (40). However, STAT6 sites have also been identified in other locations, even in introns (41). The four central thymidines of the CCL23 STAT6 site 5′-TTT TTTT GAA-3′, which separate the palindromic TTC half-sites, are not found in other STAT6 sites. An alignment of functional STAT6 sites (40) resulted in a refined consensus sequence, 5′-TTT C(T/C)(G/A)G GAA-3′. It is likely that the low-affinity binding of STAT6 to the CCL23 STAT6 site compared with the site found in the eotaxin-3 promoter is due to the differences in the variable central positions.

Downstream of the STAT6 site, another negatively acting regulatory DNA element was mapped. The presence of this sequence suppressed the IL-4 response mediated via the STAT6 site. The function of the element was not gene specific since it attenuated the IL-4-driven activation of the eotaxin-3 and the IgE germ line promoter. It will be interesting to determine whether the negative element also affects the activation of other genes which are not regulated by IL-4. Bioinformatics predicted the possible interaction of the transcription factors H-APF-1 and P3A with this sequence. H-APF-1 was originally described as liver-specific transcription factor which is involved in the IL-6-driven induction of the C-reactive protein gene promoter (42). P3A transcription factors were first described in sea urchins (43). Interestingly, these proteins exert a negative regulatory function on gene expression (44). The mechanistic interplay between the negative regulatory element and the STAT6 site in its genomic context during IL-4 treatment of monocytes needs further consideration. At least two scenarios are possible: first, the negative element is only functional in HEK293 cells due to an interacting repressor protein specifically expressed in the cell line but not in monocytes. Second, the negative function in monocytes is needed for active repression of CCL23 gene expression in the absence of cytokine stimulation. Treatment with IL-4 leads to STAT6 activation which, upon binding to its cognate site, overrides the negative effect via a factor expressed in monocytes but not in HEK293 cells. Comparative studies in monocytes and HEK293 cells are required to discriminate between these possibilities.

Besides CCL23, IL-4 and IL-13 can also regulate the expression of other chemokines, especially members of the CC chemokine subfamily. Eotaxin-1 and eotaxin-3 production has been shown to be inducible by these Th2-associated cytokines in a STAT6-dependent fashion by human fibroblasts (23–25). CCL17 and CCL22 are induced by IL-4 and IL-13 in a variety of cell types (reviewed in Ref. 45), although an involvement of STAT6 has not been described. Similarly, CCL18 can be up-regulated in monocytes and DC by IL-4 (46, 47). This type of cytokine regulation appears to be relevant for diseases in which Th2 cells and their derived cytokines play a major role, such as atopic dermatitis (48). Therefore, an association of CCL23 expression with elevated IgE levels was investigated. Increased serum IgE level is one of several parameters used to define the atopic phenotype in adults but is not an absolute criterion. Unlike CCL18 or CCL22, however, no increase in plasma levels of CCL23 in atopic individuals (as defined by elevated serum IgE > 100 kU/L) was observed. The same was true with the subgroup of plasma samples derived from individuals who were positively diagnosed for atopy irrespective of serum IgE levels (data not shown). However, increased numbers of CCL23-positive cells were found in the epidermis of skin derived from atopic dermatitis patients. Because CCL23 has been shown to have chemotactic activity toward monocytes and DC (12, 14–16), it can be speculated whether this chemokine is involved in the migration of these cell types during the course of this disease. In any case, CCL23 expression in skin may have the potential to be developed as novel biomarker for atopic dermatitis in the future.

Table I. No correlation between CCL23 and total IgE expression in human plasma

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<tr>
<th>Sample Size</th>
<th>No. of CCL23-Positive Donors</th>
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<tr>
<td>Total no. of donors</td>
<td>57</td>
</tr>
<tr>
<td>Atopic donors</td>
<td>13</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>44</td>
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* Human plasma samples were analyzed for CCL23 and total IgE. The percentages in parentheses indicate the number of CCL23-positive donors in the sample.
Disclosures
All of the authors are with Novartis Pharma AG: N. Harrer, J. M. Carballido, and M. Wöistheilschläger are full-time employees; A. Müller and C. Günther are Postdoctoral Fellows, and H. Novak is a PhD student.

References