Cutting Edge: IL-4 Production by Mast Cells Does Not Require c-\textit{maf}

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The c-maf transcription factor is selectively expressed in IL-4-producing Th2 cells. It has been implicated in IL-4 gene transcription based on its ability to directly activate the IL-4 gene in nonexpressing B cells and to promote IL-4-induced Th2 differentiation. However, it has not been definitively shown that IL-4 production by other cells is dependent on the presence of c-maf. Here, we show that IL-4-producing mast cells do not express the c-maf factor. Furthermore, mutation of a defined c-maf binding site within the proximal IL-4 promoter, which profoundly affects transcription in T cells, has no effect on expression of a reporter gene driven by the IL-4 promoter in mast cells. These results demonstrate that c-maf and its target binding site are not required for IL-4 production in all cell types and delineate additional cis- and trans-acting elements that contribute to the cell-type specific transcriptional regulation of IL-4. The Journal of Immunology, 1999, 163: 1733–1736.

The ability of a host to effectively eliminate an invading pathogen depends on the generation of appropriate effector-specific immune responses. Subsets of CD4+ T cells distinguished by their cytokine expression control this decision. Although still poorly defined, early events in an infection involving IL-4 signaling pathways direct the development of these T cell subsets and subsequent cytokine release (for review, see Ref. 1). Th2 cells, which express IL-4 as well as IL-5, IL-6, IL-10, and IL-13, are considered to be protective against extracellular parasites, can down-regulate Th1-mediated autoimmune disease, and promote allergic responses. Bystander non-B, non-T cells (2) such as mast cells and basophils, as well as T cells themselves (3) can produce IL-4 and influence the Th differentiation decision. Thus, understanding the control of IL-4 expression in all IL-4 producing cell types is of great interest.

C-maf, a member of the AP-1 family of transcription factors, has been identified as one of the factors responsible for Th2-specific expression of IL-4 (4). This conclusion is based on several observations: 1) c-maf is expressed in Th2 but not Th1 cells (4); 2) a c-maf binding site, termed MARE, is located within the IL-4 proximal promoter, and mutation of this site significantly reduces IL-4 promoter-mediated transcriptional activation (5); 3) recombinant c-maf specifically associates with this site (4); 4) cotransfection of c-maf and NF-AT expression constructs into B cells can induce ectopic IL-4 expression (4); 5) overexpression of c-maf in vivo results in a significant increase in Th2 cytokines and an inhibition of Th1 cytokines such as IFN-γ (6). Taken together, these results implicate c-maf as a critical factor in regulating the pattern of cytokine expression by T cells through its direct action on IL-4 transcription. In this report, we provide evidence that c-maf and the c-maf binding site (MARE) within the proximal IL-4 promoter does not contribute to IL-4 gene expression in mast cells.

Materials and Methods

Cell culture and stimulation

Culture conditions for CFTL15 and bone marrow-derived mast cells (BMMC), M12.4.1 B cells, P815- and ABFTL3-transformed mast cells, as well as DO11.10 Th1 and Th2 cells and Jurkat T cells have been described previously (7–10). Mast cells were stimulated with 1 µg/ml ionomycin (Calbiochem, La Jolla, CA), and T cells were stimulated with 20 ng/ml PMA and ionomycin.

RNA expression analysis

Northern analysis was performed with a c-maf-specific probe corresponding to nucleotides 2263–2680 (11) and a murine IL-4 cDNA probe corresponding to nucleotides 40–412 (12). RT-PCR was performed using the following primers: actin, TGTTACCAACTGGGACGCA (forward) and ACATCGCAGTTGCTGCTTATT (reverse); and c-maf, GTGATGCCATCTTTTGAAATTGGG (forward) and AGAGGCTGGGAAACACACAGCAAG (reverse).

Nuclear extract preparation and EMSA

CFTL15 and DO11.10 Th1 and Th2 nuclear extracts were prepared and analyzed by EMSA as described previously (13) using the following oligonucleotide probes: MARE, GGATCGAGGCGCGCGGA; STAT6 (control); TATAATGACGT(C/A)GCACGATTACT; AP-1, GACTGACGTACGT(C/A)GCACGATTACT; and c-maf, TGGATGCCATTTTTGAAATTGGG (forward) and AGAGGCTGGGAAACACACAGCAAG (reverse).
IL-4 promoter activity assays

Transient transfections of the −302 IL-4 promoter/chloramphenicol acetyl transferase (CAT) construct have been previously described (13). Briefly, 5 × 10⁵ DO11.10 Th2 cells or CFTL15 mast cells were electroporated with 20 μg of plasmid DNA at 270 V and 260 μF (T cells) or 450 V and 400 μF (mast cells) in a 0.4-cm gap cuvette using a BTX (San Diego, CA) electroporator. The next day, cells were stimulated for 24 h, and then cells were harvested and whole-cell extracts were prepared. CAT activity was determined using a liquid scintillation assay. Equal amounts of protein were used from each extract, and the results are representative of at least three experiments. Data are presented as “percent wild-type”; in T cells the cpmp for the −302 wild-type plasmid ranged from 1246 to 2306 cpmp. PCAT basic promoterless control plasmid was consistently <20% of this number. The cpms ranged between 5565 to 6425 for the −302 construct in mast cells. Mutations within the MARE binding site at −43 to −38 were generated by site-directed mutagenesis using a “Quick change” kit according to the manufacturer’s protocol (Stratagene, La Jolla, CA). Primers: wild-type IL-4 (−52 to −38), CCCTGGTTTGGCAACTTTACTC; mutant IL-4 (−52 to −38), CCCTGGTTGACACACTTTAACCT (mutant nucleotides are bold).

Results and Discussion

Mast cells do not express c-maf mRNA

It was previously shown that c-maf is expressed in Th2 clones but not Th1 clones and is induced during in vitro Th2 differentiation (4). To determine whether this factor is expressed in mast cells, Northern blot analysis was performed using RNA from several T and mast cell lines. As shown in Fig. 1A, c-maf mRNA is observed in both unstimulated EL-4 T cells and the DO11.10 Th2 line. However, no message is detectable in any mast cell line or the DO11.10 Th1 cell line. Activation of the mast cells, which induces significant amounts of IL-4 mRNA, has no effect on c-maf expression (Fig. 1B). A more sensitive RT-PCR analysis, using c-maf-specific primers, also failed to detect c-maf expression in mast cells (Fig. 1C).

The IL-4 MARE element is critical for promoter function in T cells but not mast cells

A half-MARE site is located in the IL-4 promoter adjacent to an essential NF-AT element (4, 5). This region maps to a Th2-specific footprint in the IL-4 promoter and can bind recombinant c-maf protein (4). Using high-resolution mutagenesis of this region, Hodge et al. demonstrated that the MARE sequence must be intact for inducible transcription of a CAT reporter gene driven by the IL-4 promoter (5). C-maf belongs to the maf subfamily of AP-1 proteins and can bind to the MARE sequence as a homodimer or heterodimer with other AP-1 family members (14). Although c-maf is not present in mast cells, it is possible that the MARE element acts through another related factor to control IL-4 transcription. To test this possibility, mutations were introduced in the MARE site in the context of the −302 to +5 bp IL-4 promoter fragment fused to a CAT reporter gene. This mutation disrupts c-maf binding to this site as assessed by gel shift analysis (4, 5). As shown in Fig. 2, the MARE mutation abrogates promoter activity in DO11.10 T cell lines, but has little effect on promoter function in CFTL15 mast cells.

EMSA experiments were also performed with a consensus MARE DNA probe and nuclear extracts from T and mast cells. Nuclear extracts from Th2 cells form a specific complex, whereas Th1 extracts form a much weaker and slower migrating complex (Fig. 3). However, mast cell extracts do not form specific complexes with the MARE probe. These same extracts can form complexes with the IL-4 ARE (P1) oligonucleotide probe, which binds NF-AT (8, 15) (data not shown), attesting to the integrity of the extract preparation. Together, these results indicate that mast cells do not contain MARE binding factors and that the MARE element within the IL-4 proximal promoter is not a site of IL-4 gene regulation in mast cells.

It is becoming clear that the mechanisms used by T and mast cells to control IL-4 production are different, reflecting the distinct extracellular signals that elicit IL-4 and the unique role each cell type has in the immune response. In fact, several lines of evidence indicate that mast cells are subject to less stringent control of their IL-4 gene expression than T lymphocytes. T cells, the classic “adaptive” immune cells, require three cell divisions to become fully activated and produce large amounts of IL-4 (16). In contrast, mast cells do not require priming and can induce IL-4 gene transcription within 90 min of stimulation (17). They also constitutively store IL-4 in granules that are poised for immediate release upon cell activation (18). These functional differences can be explained on a molecular level by the unique subset of transcription factors that regulate IL-4 in these two cell types. Normal mast cells do not express either c-maf, as shown here, or GATA-3 (19), demonstrating that these factors are restricted to the Th2 subset and likely ensure a controlled Th2 response. IL-4 gene expression in both T and mast cells is dependent on the inducible activation of
FIGURE 2. The MARE site is required for IL-4 promoter function in T cells but not mast cells. A, Schematic representation of a portion of the proximal regulatory region of the IL-4 promoter indicating the introduced mutations in the MARE element. B, DO11.10 Th2 cells and CFTL15 mast cells were transiently transfected with each construct, then stimulated for 24 h before CAT activity was measured. Results are representative of at least three independent experiments performed with each cell type.

NF-AT (8, 15, 20); however, there is evidence that each cell type employs distinct NF-AT isoforms (10, 15). AP-1, which comprises the NF-AT complex at the P1 site and is essential for full promoter activity in T cells, does not act at this site in mast cells. (8). IL-4 production by mast cells is also independent of STAT6 (17). Furthermore, a regulatory element in the second intron binds factors selectively expressed in mast cells including PU.1 and GATA-1 and -2 and exhibits mast cell specific activity in in vitro enhancer-reporter assays (21). Its constitutive activity in these assays may reflect its ability to confer low-level constitutive expression on the reporter assays (21). Its constitutive activity in these assays may reflect the lack of controls on early expression of IL-4 in activated mast cells, there appears to be an intrinsic mechanism to regulate late expression of this cytokine in an immune response.

Based on studies of infections in mast cell-deficient mice, it has been proposed that mast cells act as innate “sentinels” that initiate and regulate the ensuing protective response (24–26). This model is supported by data showing that mast cells can be activated by bacterial or parasitic Ags (27, 28), they can migrate to primary lymphoid organs after activation (29), and they can process and present Ags to T cells via class II MHC molecules (30). Our results fit with this model, suggesting that the rather indiscriminate release of IL-4 by mast cells early in an infection could induce or expand T cells for an effective late-phase Th2 response to pathogens.

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References

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