Chromatin Remodeling of Interleukin-17 (IL-17)-IL-17F Cytokine Gene Locus during Inflammatory Helper T Cell Differentiation

During differentiation of naive CD4+ helper T (TH) cells into effector cells, specific cytokine gene loci undergo extensive changes in chromatin modification. A novel lineage of TH cells that is regulated by transforming growth factor-β (TGFβ) and interleukin-6 (IL-6) has been identified recently as promoting tissue inflammation. These inflammatory TH (THi) cells, also called TH17 or THIL-17, produce IL-17 and IL-17F, two highly homologous cytokines that have genes located in the same chromosomal region. Here, using chromatin immunoprecipitation techniques, we have demonstrated that similar to the regulation in TH1 and TH2 cell lineages, polarization of THi cells was accompanied by selective chromatin remodeling events. Histone H3 acetylation and Lys-4 tri-methylation were specifically associated with IL-17 and IL-17F gene promoters in THi lineage. At an early stage of T cell activation, histone acetylation on these promoters was greatly promoted by a combination of TGFβ and IL-6, suggesting their synergistic role in initiating chromatin accessibility for transcription factors. Furthermore, we identified multiple noncoding sequences within the IL-17-IL-17F locus conserved across species. These elements were also associated with hyperacetylated histone 3 in a lineage-specific manner and may thus serve as potential regulatory regions. In summary, our results demonstrate for the first time that THi cell differentiation is associated with epigenetic changes in the IL-17-IL-17F locus, which suggests novel mechanisms in T cell functional regulation.

CD4+ helper T (TH)2 cells are essential organizers in immune responses. Upon activation, naive TH cells differentiate into effector cells that have been historically classified into two lineages, TH1 and TH2, based on their cytokine secretion and immune regulatory function (1, 2). TH1 cells secrete IFN-γ and regulate cellular immunity, whereas TH2 cells produce IL-4, IL-5, and IL-13 and mediate humoral responses. IL-17-producing T helper cells have been recently identified as a distinct inflammatory TH (THi) cell lineage (3, 4). THi cells produce IL-17 and IL-17F and play a critical role in regulating inflammatory responses (4–6).

Similar to TH1 and TH2 lineage differentiation, THi cell development is mediated by selective cytokine signals. It was shown that early induction of THi cells in vitro is initiated by TGFβ and IL-6 in antigen receptor-stimulated naive TH cells (7, 8). At the transcriptional level, the differentiation of THi lineage induced by cytokines requires an orphan nuclear receptor, RORγt, that is selectively expressed in THi cells (9). RORγt induces the transcription of the genes encoding IL-17 and the related cytokine IL-17F in TH cells and is required for their expression in response to IL-6 and TGFβ.

A growing body of evidence demonstrates that epigenetic regulation is another important mechanism of TH cell differentiation. Epigenetic changes mediated by covalent histone modification have been observed in a number of models of TH cells differentiation and provide a mechanism whereby cytokine gene transcriptions are stable and heritable in a polarized cell lineage. Various studies have shown that both IL-4/IL-5/IL-13/Rad50 and IFN-γ cytokine gene loci in both gene promoters and conserved noncoding sequences (CNS) undergo extensive chromatin remodeling when naive TH cells are differentiated into TH1 and TH2 cells (10).

Because THi differentiation is still poorly understood, we thus have examined the possible role of chromatin remodeling in mediating selective IL-17 and IL-17F gene expression by differentiated THi cells. We show here that under THi-polarizing conditions, histone H3 at the promoter regions of both IL-17 and IL-17F genes becomes hyperacetylated and tri-methylated at Lys-4. The initiation of histone acetylation at an early stage of TH activation is greatly promoted by the combination of TGFβ and IL-6. Moreover, we also found widespread acetylation of histone H3 associated with several CNS elements in the IL-17-IL-17F locus in polarized THi cells. This study provides a foundation for the further understanding of gene regulation associated with THi differentiation.

EXPERIMENTAL PROCEDURES

TH Differentiation—Naive TH cells, isolated from OT-II TcR transgenic mice by AutoMACS (Miltenyi Biotec) selection of CD4+ T cells following the manufacturer’s instruction, were cultured as described previously (11) at a ratio of 1:1 with irradiated B6 splenic antigen-presenting cells depleted of T cells along with 10 μg/ml chicken ovalbumin (OVA) peptide (amino acids 323–339; ISQAVHAAHAEINEAGR). Under THi condition, cells were incubated in the presence of 5 ng/ml TGFβ, 10 ng/ml IL-6, 10 ng/ml IL-23, 10 ng/ml IL-1β, 10 ng/ml TNFα, 10 μg/ml anti-IL-4 (11B11), and 10 μg/ml anti-IFN-γ (XMG1.2).
Under TH1 condition, cells were incubated in the presence of 10 ng/ml IL-12, 10 ng/ml IFN-γ, and 10 μg/ml anti-IL-4. Under TH2 condition, cells were incubated in the presence of 10 ng/ml IL-4 and 10 μg/ml anti-IFN-γ. The medium was changed when necessary, but the concentrations of cytokines and antiboies were maintained. Differentiated cells were analyzed 5 days after activation.

ChIP Assay—ChIP assays were carried out as described (12). Cells were cross-linked using formaldehyde, and the nuclei were isolated and sonicated, DNA-protein complexes were immunoprecipitated with protein A-Sepharose pre-blocked with salmon sperm DNA using anti-acetylated histone H3 Ab (Upstate, Lake Placid, NY; catalog no. 06-599) and anti-trimethylated histone H3-K4 Ab (Upstate, Lake Placid, NY; catalog no. 07-473). After washing, elution, and reversion of cross-links, the DNA was isolated and used in radioactive PCR reactions. The primers used for PCR analysis were: IL-4P, 5′-CCCCGGTGTTCTATCCGCCAGC-3′ and 5′-GACCGCCAGAGGACTACCTTG-3′; IL-17P, 5′-CATATCGAGGGTGTCGGA-3′; IFN-γP, 5′-CGTAATCCCGAGGAGCAGCC-3′; and 5′-CATATTCTTCGTTGTGC-3′. The DNA fragments obtained were separated by 12% PAGE (left), and the signal intensity was quantified and normalized to β-actin promoter (right). Data are representative of at least four independent experiments with similar results.

RESULTS AND DISCUSSION

THI Differentiation Is Accompanied by Selective Chromatin Changes within the Cytokine Gene Promoters—To determine whether epigenetic changes occur during THi cell development, we differentiated OT-II cells toward TH1, TH2, and THi lineages. 5 days later, differentiated cells were harvested, and a ChIP assay was performed using antibodies against acetylated histone H3 or tri-methylated Lys-4 of histone H3. Consistent with the literature (10), in TH1 cells, histone H3 associated with the IL-4 gene promoter was hypoacetylated and the IL-17 and IL-17F promoter regions were hypoacetylated in histone H3. Conversely, in TH2 cells, histone H3 associated with the IL-4 gene promoter was highly acetylated, and at the IFN-γ gene promoter it exhibited very low levels of acetylation (Fig. 1A). The histone H3 modifications associated with the IL-17 and IL-17F promoter regions in different TH subsets are depicted in Fig. 1B. The THi cell lineages were hyperacetylated at the IL-17 and IL-17F gene promoters, thereby silencing these genes in TH1 and TH2 subsets. However, as a result of THi lineage differentiation, histone H3 acetylation at the IL-17 and IL-17F gene promoters was greatly increased (Fig. 1A), in keeping with the ability of THi cells to produce significant amounts of IL-17 and IL-17F.
cytokines. Unlike the IL-17 and IL-17F loci, histone H3 within the IL-4 and IFN-γ gene promoter regions was hypoacetylated in THi cells.

We also examined the tri-methylation at Lys-4 in H3 (K4me3), another form of histone modification associated with actively transcribed chromatin structure. As shown at IL-17F gene promoters was triggered by the combination of IL-6 and TGFβ 48 h after activation (Fig. 2B). Therefore, it is likely that at the early stage of TH cells activation, IL-6 and TGFβ together control the accessibility of the IL-17-IL-17F gene locus to allow the binding of transcription factors and promote the further polarization of T cells.

Thus, our results indicate that THi cell development is accompanied by epigenetic changes at both IL-17 and IL-17F gene promoters. This suggests that similar to other T helper cell lineages, epigenetic modification is an integral part of THi cell differentiation.

Cytokine Induction of Chromatin Remodeling at the IL-17-IL-17F Gene Promoters—Although recent data indicate the essential function of TGFβ and IL-6 in THi differentiation (7, 8), the underlying molecular mechanisms are unclear. We thus examined the induction of IL-17 and IL-17F gene transcription in naive TH cells upon plate-bound CD3 and CD28 stimulation in the presence of various cytokines. Treatment with IL-6 resulted in significant increase of both IL-17 and IL-17F gene transcripts 2 days after activation, as measured by quantitative RT-PCR, whereas TGFβ alone had no effect (Fig. 2A). On the other hand, the combination of IL-6 and TGFβ led to an even more potent induction of IL-17 and IL-17F mRNA levels (Fig. 2A), which indicates a synergistic function of the two cytokines in IL-17 and IL-17F gene transcription.

Next, to assess whether the induction of IL-17 and IL-17F gene transcription is associated with chromatin remodeling, we examined histone H3 acetylation levels in naive TH cells upon various cytokines treatments. Consistent with the above observations, H3 hyperacetylation at the IL-17 and TGFβ led to an even more potent induction of IL-17 and IL-17F mRNA levels (Fig. 2A), which indicates a synergistic function of the two cytokines in IL-17 and IL-17F gene transcription.
Lineage-specific Histone H3 Hyperacetylation at Multiple CNS—The genes encoding IL-17 and IL-17F cytokines are located on mouse chromosome 1 flanked by the non-cytokine genes MCM3 and PKHD1 (Fig. 3A). To further characterize the epigenetic changes associated with THi cell differentiation, we performed comparative genomic analysis to identify CNS. Using the on-line-based mVISTA alignment program, we compared DNA sequences of the IL-17-IL-17F loci among mouse, dog, and human. Regions containing fragments longer than 100 bp, with at least 75% homology between paired sequences, were identified as CNS. We found eight CNS in this region (Fig. 3A), which may represent potential regulatory elements.

Previous studies have demonstrated that histone acetylation is often colocalized with CNS (13). To evaluate the function of these CNS, we performed a ChIP assay using naive or differentiated TH1, TH2, and THi cells. Naive CD4 T cells exhibited merely background levels of H3 acetylation at all of CNS sites (Fig. 3B). Following THi differentiation, most of the CNS analyzed exhibited lineage-specific hyperacetylation of histone H3. Although the CNS-5 site remained acetylated in all types of polarized T helper cells, we did not detect significant levels of acetylated histone H3 in TH1 or TH2 cells at seven of eight CNS sites. The nonconserved region between CNS-1 and CNS-2, chosen as a negative control, exhibited no association with acetylated histone H3 in all cell samples (data not shown). These results suggest that the majority of CNS located within this locus undergo association with hyperacetylated H3 during THi cell development. Therefore, the chromatin remodeling appears to be spread though the entire locus, providing accessibility of the IL-17/IL-17F gene promoter regions and other regulatory elements.

Our data from the current study demonstrate that similar to other TH subsets, the process of pro-inflammatory T cell differentiation is tightly associated with stable epigenetic modifications at THi-specific cytokine locus and possibly results in long-range coordinated chromatin remodeling. Activation of IL-17 and IL-17F genes by cytokine signals appears to be functionally correlated with histone H3 hyperacetylation. Moreover, we also found widespread H3 acetylation associated with most CNS in the IL-17-IL-17F locus. These observations not only support THi as a distinct cellular lineage but also provide the basis for the further identification and characterization of putative regulatory elements involved in the regulation of THi cytokine gene expression and the selective transcription factors that target these sites.

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