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Activated Human T Cells Accomplish MHC Class II Expression Through T Cell-Specific Occupation of Class II Transactivator Promoter III

Tjadine M. Holling, Nienke van der Stoep, Edwin Quinten, and Peter J. van den Elsen

Activated human T cells express HLA-DR, HLA-DQ, and HLA-DP on their surface, but the regulation and functioning of MHC class II molecules in T lymphocytes are poorly understood. Because the MHC class II transactivator (CIITA) is essential for MHC class II expression, we have investigated transcriptional activation of CIITA in activated T cells. In this study, we show that in human activated CD4+ T cells, CIITA promoter III (CIITA-PIII) drives the expression of CIITA. The in vivo genomic footprint analysis revealed activated T cell-specific occupation of CIITA-PIII. Subsequent EMSA analysis of several promoter regions showed differences in banding pattern among activated T cells, naïve T cells, primary B cells, and Raji B cells. Activating response element (ARE)-I is shown to interact with the acute myeloid leukemia 2 transcription factor in nuclear extracts derived from both T and B cells. Interestingly, the acute myeloid leukemia 3 transcription factor was bound in nuclear extracts of T cells only. The ARE-2 sequence is able to bind CREB/activating transcription factor family members in both T and B cells. In addition, a yet unidentified Ets family member was found to interact with site C in activated T cells, whereas in B cells site C was bound by PU.1 and Pip/IFN regulatory factor 4/IFN consensus sequence binding protein for activated T cells. In Jurkat T cells, both ARE-1 and ARE-2 are crucial for CIITA-PIII activity, similar to Raji B cells. The differential banding pattern in in vivo genomic footprinting and transcription factor binding at the ARE-1 and site C between T cells and B cells probably reflects differences in CIITA-PIII activation pathways employed by these cell types. The Journal of Immunology, 2002, 168: 763–770.

M ajor histocompatibility complex class II molecules play a central role in the control of the adaptive immune response by, among others, binding and presenting immunogenic peptides to CD4+ Th cells (1). Only a limited number of cell types express MHC class II molecules; professional APCs, such as B cells, macrophages, Langerhans cells, and dendritic cells, display constitutive expression of MHC class II, while nonprofessional APCs, such as fibroblasts and T cells, acquire MHC class II expression under certain circumstances.

Much of our understanding on the transcriptional control of MHC class II and class I gene expression has been derived from studies with cells obtained from patients with a MHC class II deficiency, also referred to as bare lymphocyte syndrome (2–5). In bare lymphocyte syndrome patients, the genetic defect resides in the genes encoding transacting regulatory proteins required for transcriptional activation of MHC class II and class I promoters, i.e., in the genes encoding the MHC class II transactivator (CIITA) and the individual components of the regulatory factor X (RFX) complex (6–10). RFX manifests its role in transactivation through binding to the X1 box of the SXY regulatory module within the promoters of MHC class II genes (11). As such, RFX is crucial for the formation of a multimeric transcription factor protein/DNA complex with the CREB/activating transcription factor (ATF) proteins and the heterotrimeric complex NF-Y, which bind to the conserved X2- and Y-box promoter elements, respectively (12–14). CIITA functions as a coactivator by interacting with almost all of the components of this multimeric transcription factor complex bound to the SXY regulatory module, and together these regulatory proteins form a stable enhanceosome that drives transcription of MHC class II genes (15–19). This is achieved through interaction of the NH2-terminal activation domain of CIITA with components of the basal transcription initiation apparatus and by facilitation of chromatin remodeling through interactions with general coactivators that possess histone acetyltransferase activities (20–23).

Whereas the DNA-binding components of the enhanceosome (including RFX) are ubiquitously expressed, CIITA is only constitutively expressed in professional APCs and is induced in nonprofessional APCs. The expression of CIITA is controlled by four separate promoters, each coding for distinct first exons (24). CIITA promoter (CIITA-P) is the promoter used in dendritic cells, and CIITA-PII is expressed at insignificant levels and is as yet functionally poorly understood. CIITA-PIII is constitutively expressed in B lymphocytes and can drive CIITA expression after IFN-γ stimulation in a number of different cell types, including endothelial cells and fibroblasts (25). While the B cell-specific expression of CIITA requires a small region directly upstream of the initiation codon, the IFN-γ induction requires an additional region located ~5 kb upstream of the transcriptional start site (25).
CIITA-PIV is the principal IFN-γ-inducible promoter (24–29). The cellular and temporal diversity in MHC class II expression is thus regulated via the differential usage of the CIITA promoters.

Recently, several functional domains in CIITA-PIV and CIITA-PIII have been characterized. For the IFN-γ-mediated induction of CIITA-PIV cooperative binding of Stat1 and upstream stimulatory factor-1 to an IFN-γ activation site-like element, and binding of IFN regulatory factor 1 to an IFN-stimulated response element-like sequence in CIITA-PIV, are critical (25, 27, 28). Functional analysis of regulatory factor 1 to an IFN-stimulated response element-like site in CIITA-PIV suggested a model for the regulation of MHC class II molecules on the cell surface. This MHC class II expression is driven by CIITA-PIIII only. Notably, despite the shared usage of CIITA-PIII, differences in CIITA-PIII occupancy were observed between activated T cells and Raji B cells. Furthermore, EMSA studies showed differences in binding pattern in activated T cells and Raji B cells. This probably reflects the differences in CIITA activation pathways employed by T cells (induced expression) and Raji B cells (constitutive expression).

Materials and Methods

Cell culture

The Jurkat cell line was purchased from the American Type Culture Collection (Manassas, VA; Jurkat clone E6-1, TIB-152) and cultured in RPMI 1640 (Life Technologies, Breda, The Netherlands) supplemented with 10% heat-inactivated FBS (Greiner, Alphen a/d Rijn, The Netherlands), 10 U/ml streptomycin, 100 U/ml penicillin, and 2 mM l-glutamine.

Naïve CD4⁺ T cell isolation and stimulation

Peripheral blood was obtained from normal healthy donors by venipuncture procedure, and PBMCs were isolated using a Ficoll gradient (Pharmacia, Uppsala, Sweden). PBMCs were stained with FITC-coupled HLA-DR (1:8) and PE-coupled CD4 (1:32) Abs (both from BD Biosciences, Mountain View, CA) in PBS containing 5% heat-inactivated FBS (Greiner, Alphen a/d Rijn, The Netherlands), 100 IU/ml Penicillin, 100 U/ml Streptomycin, and 2 mM l-glutamine. Naïve CD4⁺ T cells were isolated from Ficoll-purified PBMCs using the AutoMACS T cell enrichment columns (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions.

RNA isolation and RT-PCR analysis

Total RNA was isolated from the different T cell populations using the RNAzol extraction method (Cinna/Biotecx Laboratories, Houston, TX). RNA samples (2 μg) were transcribed into cDNA using avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI). The cDNA was subsequently amplified using specific primers for CIITA-PI, CIITA-PIII, CIITA-PIV, and GAPDH: CIITA-PI, sense 5'-GGTTGAGG GAGAATCGGAGACCCCTGGT-3'; CIITA-PIII, sense 5'-GATTTCTTACATGATTGAGACCCCTGGT-3'; CIITA-PIV, sense 5'-AGCTGCGCGCGGGAGGAGACCCCTGGT-3'; CIITA-PIII, antisense 5'-CATACTGCTGATGTTGAGACCCCTGGT-3'; CIITA-PIV, antisense 5'-CATGCTGCTGATGTTGAGACCCCTGGT-3'; GAPDH sense, 5'-GGACAGGAGCGGCTGCTGATGTTGAGACCCCTGGT-3'; and GAPDH antisense, 5'-ATGACGCGCGCGGGAGGAGACCCCTGGT-3'.

One microliter of cDNA was amplified in 1× PCR buffer II (Roche Diagnostics, Mannheim, Germany), 0.3 mM NucleiX Plus PCR nucleotide mix (Amersham Pharmacia Biotech, Piscataway, NJ), 0.4 μM of both sense and antisense primers, 2.5 U AmpliTaq DNA polymerase (Roche), and MgCl₂ (Roche) in a total volume of 50 μl. The final concentration of MgCl₂ was for 1.25 mM CIITA-PI, 1.5 mM CIITA-PIII, 1.5 mM CIITA-PIV, and 4 mM GAPDH. The PCR conditions used for CIITA-PI, CIITA-PIII, and CIITA-PIV were as follows: denaturation for 1 min at 94°C, annealing for 1 min at 61°C, and extension for 1 min at 72°C for a total of 35 cycles; the PCR conditions used for GAPDH were as follows: denaturation for 1 min at 94°C, annealing for 1 min at 60°C, and elongation for 1 min at 72°C for a total of 25 cycles. The PCR products were visualized on an ethidium bromide-stained agarose gel, and colors were reversed for reasons of clarity.

EMSA

Primary B cells were harvested from human spleen using Dynabeads CD19 plus B (Dynal Biotech, Oslo, Norway), and further propagated in RPMI 1640 (Life Technologies) supplemented with 10% human serum, 10 U/ml IL-2, 100 U/ml streptomycin, 100 U/ml penicillin, and 2 mM l-glutamine.

Isolation of human primary B cells

Primary B cells were isolated from human spleen using Dynabeads CD19 plus B (Dynal Biotech, Oslo, Norway), and after positive selection the beads were removed from the cells by DETACHABead CD19 (Dynal Biotech) according to the manufacturer’s instructions.
nucleotides are underlined. Due to the limited amount of in vivo activated T cell nuclear extract, we performed the EMSA with site A and site B oligonucleotides only with nuclear extract from in vitro activated T cells, as these elements were found not to be critical for CIITA-PIII activation in Jurkat T cells.

Supershift assays were performed by incubating nuclear extract and oligonucleotide for 30 min on ice, after which 1 μg of Ab was added and incubation was continued for an additional 60 min on ice. The following Abs were used for supershift assays: ATF/CREB (sc-270) reactive with ATF-1; p-35, CREB-1 p43, and cAMP responsive element modulator-1; CREB-1 (sc-271) reactive with CREB-1 p43; ATF-1 (sc-243) reactive with ATF-1 p35; PU.1 (sc-6509); all obtained from Santa Cruz Biotechnology, Santa Cruz, CA), acute myeloid leukemia (AML)1 (PC284); AML2 (PC286); AML3 (PC287) (all obtained from Oncogene Research Products, San Diego, CA).

Reporter and expression constructs

The pGL3-CIITA-PIIII reporter construct was generated by cloning a 668-bp region of pGL2-CIITA-PIII, which contains the sequence −545 to +123 (obtained by J. P.-Y. Ting, University of North Carolina, Chapel Hill, NC) into the pGL3-basic luciferase reporter plasmid (Promega). The pGL3-CIITA-PIV construct was previously described by van den Ensen et al. (42). Several 5′ truncations of CIITA-PIII were generated. The −145 to +123 bp or the −113 to +123 bp fragment of CIITA-PIII was created by PCR using the GL2 primer (Promega) and either the pIII-145 sense primer (5′-AAATTTTAACAGAAACTGCGGCTTGGG-3′) and pGL3-CIITA-PIII as template. The PCR products were subsequently cloned into the pGL3-basic luciferase reporter plasmid (Promega). Mutations in ARE-1, ARE-2, and site C were introduced by site-directed mutagenesis using overlapping extension PCR (43). Mutation primers for the PU.1 site in site C are described above. Mutation primers for the AML site at ARE-1 were as follows: AMLM sense, 5′-CTTAAGGAGGAGCTTAATTTTAAGCTTCTCCCTTAAG-3′. Mutation primers for ARE-1 were as follows: ARE-1M sense, 5′-GGGCTTAAGCTGTAGTTGAAGAAATT-3′; ARE-1M antisense, 5′-AATTTTACCCGCTAGCTTTTGGGCC-3′. Mutation primers for the CRE site in the ARE-2 binding site were as follows: ARE-2M sense, 5′-GAGGTTTTTTGACCGGGTCCTACTGTTTTC3′; ARE-2M antisense, 5′-GAAACAAGTAGGGGCCGTCAAAAACTC-3′; the changed nucleotides underlined. All constructs were verified by DNA sequencing at the Leiden Genome Technology Center (Leiden, The Netherlands).

Transient transfections

The human T lymphocyte cell line Jurkat was transfected by electroporation (Genepulser; Bio-Rad, Richmond, CA) at 250 V and 960 μF, and harvested after 72 h. Electroporations were performed with 8 × 10^6 cells and 10 μg of luciferase pGL3-reporter construct together with 2.5 μg of SV40-Reporter and expression constructs (Promega) as an internal control. Luciferase and Renilla luciferase activity were measured using the Dual Luciferase Assay kit according to the manufacturer (Promega) and normalized for tranfection efficiency with the Renilla luciferase activity.

Results

CIITA promoter usage in activated CD4+ T cells

First we have investigated which of the CIITA promoters is used to drive CIITA expression in CD4+ HLA-DR+ T cells. Two populations of CD4+ HLA-DR+ T cells were obtained, which we designated in vivo activated CD4+ T cells and in vitro activated CD4+ T cells. In vivo activated CD4+ T cells were obtained by gating CD4+ HLA-DR+ from PBMC, and to increase cell numbers these cells were propagated in vitro using PHA, IL-2, and irradiated allogenic PBMCs. In vitro activated CD4+ T cells were derived from CD4+ HLA-DR+ gated PBMC and stimulated in vitro using CD3 and CD28 Abs, IL-2, and irradiated allogenic PBMCs. To detect which CIITA promoter was used in activated T cells, a RT-PCR with primer sets specific for CIITA-PII, CIITA-PIII, and CIITA-PIV was applied. The results of these analyses showed that both the in vivo and the in vitro activated CD4+ T cell populations employ CIITA-PIII only (Fig. 1). This implies that CIITA-PIII is thus not exclusively used in B cells but is also the principal promoter employed in activated T cells.

In vivo promoter occupation of CIITA-PIII in activated T cells

To compare promoter occupation between naive and activated T cells, and between activated T cells and Raji B cells, in vivo genomic footprinting (IVGF) analysis was employed to visualize the protein/DNA interactions on CIITA-PIII. The lower strand from nucleotide −250 to +10 of the CIITA-PIII promoter was analyzed using dimethyl sulfate as a modifying agent. Analysis of equal amounts of methylated genomic DNA by IVGF analysis showed clear differences in promoter occupation between naive and activated T cells (Fig. 2A, summarized in Fig. 2B). In the screened region, naive T cells have no protection, as its footprint pattern is comparable with that of Jurkat DNA, in which the proteins were removed before methylation (see Fig. 2A, in vitro methylated DNA).

In activated T cells, many protected guanine residues were found. Five of these protected areas were already described for the B cell line Raji (31). Similar to Raji B cells, a strong protection is seen at the ARE-1 in activated T cells (nucleotide −132 to −143). Although strong protection is seen in both activated T cells and Raji B cells at the ARE-1 binding site, several differences in the protection pattern were also found. In Raji B cells, all guanine residues in the ARE-1 binding site are protected, while in activated T cells the guanine at position −134 is not protected. Notably, the guanine residues downstream of the ARE-1 site (−124 to −89) are completely or partially protected in activated T cells. Furthermore, in activated T cells, a strong hypermethylation is seen in an area devoid of guanine residues directly downstream of the ARE-1 site; however, the significance of this nonguanine enhancement is not clear.

A second strong hypermethylation in activated T cells is seen at the ARE-2. Again the protection pattern at the ARE-2 site differs between activated T cells and Raji B cells; nucleotide −64 is enhanced in activated T cells, while this guanine residue is partially protected in Raji B cells. Furthermore, the guanine at position −61 is enhanced in Raji B cells, while in activated T cells this guanine is neither enhanced nor protected.

A third region with strong protection in activated T cells is seen between site A (nucleotide −13 to −25) and site B (−38 to −45). At site A, a cluster of four protected guanine residues is found in activated T cells, while in Raji B cells only two guanine residues are partially protected. Moreover, directly downstream of site B, protected guanine residues were detected in activated T cells. Also, we found a few protections or enhancements of promoter DNA...
upstream of the ARE-1 binding site and at site C. Overall, the CIITA-PIII in activated T cells shows more protected guanine residues outside the described Raji B cell binding regions, and within these areas the protection pattern differs between activated T and Raji B cells.

Transcription factor binding to CIITA-PIII in T cells

The IVGF analysis showed that the protection pattern at several CIITA-PIII binding sites differs between activated T cells and Raji B cells. This difference in protection pattern might be due to the binding of different transcription factors to these regulatory elements in activated T and Raji B cells. To determine which transcription factors bind to the different regulatory elements, EMSA with DNA sequences of these elements was utilized. Naive T nuclei possess low numbers of nuclear proteins (approximately seven times fewer proteins than activated T cells), which makes it difficult to observe qualitative differences. Therefore, equal amounts of nuclear proteins were used in the assays described below instead of equal numbers of cells.

ARE-1

Incubation of an oligonucleotide containing the ARE-1 binding site and nuclear extract from naive T cells, in vivo activated T cells, primary B cells, or Raji B cells revealed a complex, complex 1, specifically present in activated T cells (Fig. 3, lane 6, arrow 1), and a complex, complex 2, specifically present in naive T cells and Raji B cells (Fig. 3, lanes 1 and 11, arrow 2). This complex was difficult to detect in primary B cells. No differences were found in banding pattern between in vivo and in vitro activated T cells (not shown). The ARE-1 site displayed a high homology with the binding site for proteins of the AML/core-binding factor (CBF) family. In a supershift assay, addition of the AML1 Ab did not reveal the presence of AML1 in the complex (Fig. 3, lanes 3, 8, 13, and 17), whereas AML2 and AML3 were readily detected. Addition of AML2 Ab shifted complex 1 in activated T cells (Fig. 3, lane 9, from arrow 1 to arrow 2*). Moreover, in naive T cells and Raji B cells, complex 2 is also shifted with this Ab (Fig. 3, lanes 4 and 14, from arrow 2 to arrow 2*). These two different AML2 complexes might reflect the presence of an isoform of AML2 or an additional factor in the complex. Inclusion of the AML2 Ab also resulted in the formation of an additional complex in primary B cells. Interestingly, addition of the AML3 Ab to the binding reaction resulted in the formation of a large complex in naive and activated T cells, but not with primary B cells or Raji B cell nuclear extract (Fig. 3, lanes 5, 10, 15, and 19, arrow 3*). However, it is not clear which DNA-protein complex is shifted by the AML3 Ab. Thus, AML3 binds the ARE-1 site in naive and activated T cells, but not in B cells, and a specific AML2/ARE-1 complex is formed in activated T cells.

ARE-2

Next, an oligonucleotide spanning the ARE-2 binding site was used to test transcription factor binding to this site. Three specific complexes compared with Jurkat T cell in vitro methylated DNA. Open arrowheads indicate protected residues in activated T cells, while filled arrowheads indicate hypermethylation. Earlier described regions are indicated on the left. B, Schematic representation of DNA-protein interactions in T and Raji B cells. Earlier described regions are boxed. Open triangles (protection) and filled triangles (hypermethylation) indicate found DNA-protein interactions in activated T cells. Open circles (protection) and filled circles (hypermethylation) indicated found DNA-protein interactions in Raji B cells. Gray circles indicate residues earlier reported to be protected in Raji cells (31).
were seen using the nuclear extracts of naive T cells, in vivo activated T cells, Raji B cells, and primary B cells. Normal rabbit serum (NRS) or polyclonal Abs to AML1, AML2, and AML3 were added, as indicated above each lane. In activated T cells, a specific AML2 complex was found (arrow 1), which differs in size with AML2 complexes found in naive T cells and Raji B cells (arrow 2). The AML2 Ab showed a supershift in naive T cells, activated T cells, Raji B cells, and primary B cells (arrow 2*). The AML3 Ab supershifted an unidentified complex in T cells, but not in Raji B cells and primary B cells (arrow 3*).

Sites A and B
The site A sequence formed a complex (Fig. 5A, arrow 1) with a factor predominantly present in in vitro activated T cells. A more slowly migrating complex (Fig. 5A, arrow 2) can be seen using Raji B cell nuclear extracts, but not in an activated T cell extract. This later complex was previously suggested to be a NF-1 (related) transcription factor (31). Indeed, an oligonucleotide containing the consensus NF-1-binding sequence was able to eliminate the upper complex in Raji B cells (Fig. 5A, arrow 2), but not the lower complex in T cells (Fig. 5A, arrow 1; data not shown).

Site B is able to form several complexes in in vitro activated T cells, which differ from those in naive T cells and in Raji B cells (Fig. 5B, bracket). The sequence of site B displays weak homology with an octamer factor-binding site. However, it was shown previously that only very weak binding of octamer-binding transcription factor-1 could be detected at site B (31). Because site B has no further apparent homology with any transcription factor-binding site known to date, it remains unclear which proteins bind to site B in activated T cells and Raji B cells.

Site C
Incubation of a nuclear extract from in vivo and in vitro activated T cells with an oligonucleotide containing the sequence of site C resulted in the formation of a specific activated T cell complex (Fig. 6, lane 2, arrow 1; and data not shown), which is not detectable in nuclear extracts from naive T cells or Raji B cells (Fig. 6, lanes 1 and 3). Furthermore, in Raji B cells, two specific complexes are formed (Fig. 6, lane 3, arrows 2 and 3). Site C has a high homology, with the binding site of transcription factors belonging to the Ets family. An oligonucleotide containing site C with a mutated Ets binding site was not able to form both complex 1 in activated T cells and complex 3 in Raji B cells (Fig. 6, lanes 7 and 10), indicating that these complexes are formed by Ets motif-binding factors. Because the Ets protein containing complex 1 is
In the presence of PU.1 or Pip-specific T cells are able to bind to site C. Incubation of wild-type site regulatory factor 4/IFN consensus sequence binding protein activated the Ets family member PU.1 and its activating partner Pip/IFN nuclear extract. Furthermore, in Raji B cells, we now demonstrate results in the same banding pattern with naive and activated T cell formed only in activated T cells, mutation of this Ets binding site.

Site C is an Ets binding site in T and B cells. EMSAs were performed as indicated in Fig. 3, except that the used oligonucleotide contained either the wild-type site C sequence or the site C sequence with a mutated Ets binding site. Abs recognizing PU.1 or Pip were added as indicated above each lane. In in vivo activated T cells (active T), a specific Ets motif-binding factor was found (arrow 1). In Raji B cells, two specific complexes are formed (arrows 2 and 3), and adding PU.1 or Pip Abs reduced the formation of these complexes.

formed only in activated T cells, mutation of this Ets binding site results in the same banding pattern with naive and activated T cell nuclear extract. Furthermore, in Raji B cells, we now demonstrate that the Ets family member PU.1 and its activating partner Pip/IFN regulatory factor 4/IFN consensus sequence binding protein activated T cells are able to bind to site C. Incubation of wild-type site C in the presence of PU.1 or Pip-specific Abs showed that complex 2 is formed by Pip (Fig. 6, lane 11) and complex 3 by PU.1 (Fig. 6, lane 12). In accordance with the fact that PU.1 is not expressed in T cells, the PU.1 Ab was not able to change the binding pattern in T cells (Fig. 6, lane g). Thus, in Raji B cells PU.1 and Pip are capable of binding to site C, while in activated T cells site C probably binds an Ets-like protein.

CIITA-PIII activity in Jurkat T cells

To correlate protein/DNA interactions with CIITA-PIII transcriptional activity in T cells, several 5’ truncated promoter constructs and mutant promoter constructs were generated and transiently transfected in the Jurkat T cell line (Fig. 7). The activity of these truncated and mutant CIITA-PIII promoter constructs was compared with the activity of a wild-type CIITA-PIII promoter fragment encompassing nucleotides −545 to +123. The activity of CIITA-PIII-145, lacking site C, was about 75% of the wild-type CIITA-PIII promoter activity. Mutation of the PU.1 site in wild-type CIITA-PIII site C results in a decreased CIITA-PIII activity to 60%, which is comparable with the reduction in activity seen with CIITA-PIII-145. Deletion of the promoter region containing both site C and ARE-1 in CIITA-PIII-113 caused a dramatic drop in promoter activity to 18%. When in wild-type CIITA-PIII the AML-binding sequence at ARE-1 was mutated, promoter activity decreased to 38% (not shown). Moreover, mutation of the entire ARE-1 site in wild-type CIITA-PIII completely abolished CIITA-PIII activity. Also, mutation of the CRE site at the ARE-2 annihilated CIITA-PIII activity, revealing that both ARE-1 and the ARE-2/CRE site are of crucial importance for CIITA-PIII promoter activity in Jurkat T cells.

Discussion

To gain more insight into MHC class II expression in T lymphocytes, we have investigated the regulation of the MHC CIITA in activated human T cells. Naive T cells do not express MHC class II molecules on their cell surface (44), congruent with the lack of CIITA transcripts in this T cell population (Fig. 1). Moreover, naive T cells displayed an almost bare CIITA promoter occupancy, as analyzed by IVGF (Fig. 2A). This lack of transcription factor binding might relate to the low amount of nuclear protein per naive T cell. However, when in the EMSA analysis equal amounts of nuclear protein were used, different banding patterns were observed in naive T cells and activated T cells with ARE-1, and site A, B, and C oligonucleotides (Figs. 3, 5, and 6). Thus, naive and activated T cells not only differ from each other with respect to the amount of nuclear protein per cell, but also differ qualitatively with respect to the proteins able to interact with the various regulatory elements.

It was found that of the four CIITA promoters, only CIITA-PIII is used to drive CIITA expression in both in vivo and in vitro activated T cells. CIITA-PIII is thus, besides by B cells, also employed by activated T cells. However, B cells have constitutive CIITA expression, while in activated T cells CIITA expression is induced, suggesting the presence of alternative activation pathways of CIITA-PIII. In accordance, IVGF analysis revealed differences in promoter occupation between activated T cells and Raji B cells. A prominent difference was seen in promoter occupation at the ARE-2 site. For Raji B cells, it has already been shown that the ARE-2 element is a binding site for members of the CREB/ATF family of transcription factors (N. Van der Stoep, E. Quinten, and P. J. van den Elsen, unpublished observations). We now show that in activated T cells CREB/ATF family members also can bind the ARE-2 element of CIITA-PIII. Although the promoter occupancy differs at the ARE-2 site between activated T cells and Raji B cells, EMSA analysis did not reveal any differences in the specific banding pattern between these cell types. On the one hand, it
is possible that the conditions used in our EMSA analysis did not support the formation of protein/DNA complexes that occurs in activated T cells in vivo. On the other hand, the IVGF analysis showed that CIITA-PIII upstream of ARE-2, in contrast to Raji B cells, is highly occupied in activated T cells. Factors binding to this region might interact with CREB/ATF at the ARE-2 site, causing differences in protection pattern in the genomic footprint compared with Raji B cells, or, alternatively, occupation of this region leads to a conformational change of the DNA and to a change in methylation pattern. Besides in activated T cells and Raji B cells, in primary B cells and naive T cells CREB/ATF binding also was demonstrated with the ARE-2 sequence. Yet, because the IVGF analysis revealed that the ARE-2 is not occupied in naive T cells, these data suggest that in naive T cells CREB/ATF is already present in very low amounts in the cell but is recruited to ARE-2 following T cell activation. This might be achieved through the activities of coactivators and/or, as discussed above, by the increased amounts of CREB/ATF in the nuclei of activated T cells.

Also, differences in promoter occupation between B and T cells were found at the ARE-1 site. The ARE-1 site in CIITA-PIII was proposed to be the binding site of a transcriptional enhancer factor-2-like transcription factor, as suggested by competition with a transcriptional enhancer factor-2 consensus oligonucleotide (31). Using specific Abs, we now show that the ARE-1 sequence can be bound by AML2, and that the binding pattern differs among activated T cells and naive T cells, primary B cells, or Raji B cells. It suggests that in activated T cells ARE-1 is bound by an AML2 isoform or, alternatively, that AML2 is complexed at ARE-1 with additional factors in naive T cells and Raji B cells. Notably, we also show that binding of AML3 to the ARE-1 site was specific for T lymphocytes because no AML3 Ab supershift is observed with nuclear extracts of Raji B cells or primary B cells.

All members of the AML family are capable of forming a heterodimer with the partner subunit CBFB and form the CBFB complex. Cooperative binding of CBFB and Ets-1 to their separate binding sites has been demonstrated (45, 46), and this cooperative binding is relatively independent of the orientation and distance between the two binding sites (45). In this respect, it is of interest to note that CIITA-PIII contains both the CBFB and an Ets binding site. An AML binding site has been located next to ARE-1 and additional factors in naive T cells and Raji B cells. Notably, we also show that binding of AML3 to the ARE-1 site was specific for T lymphocytes because no AML3 Ab supershift is observed with nuclear extracts of Raji B cells or primary B cells.

In conclusion, CIITA-PIII is the principal CIITA promoter employed by human activated T cells to drive expression of CIITA. While the IVGF analysis of naive T cells showed no transcription factor binding on CIITA-PIII, several DNA/protein interactions were found to be specific for activated T cells compared with Raji B cells. In accordance, using band shift assays, differences in factor binding were found among activated T cells, naive T cells, primary B cells, and Raji B cells at established regulatory sites. Some of these DNA binding factors were identified as CREB/ATF, AML/Runx, and Ets family members.

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