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Human GATA-3: a lineage-restricted transcription factor that regulates the expression of the T cell receptor α gene

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In addition to its role in the recognition of foreign antigens, the T cell receptor (TCR) α gene serves as a model system for studies of developmentally-regulated, lineage-specific gene expression in T cells. TCR α gene expression is restricted to cells of the TCR α/β+ lineage, and is controlled by a T cell-specific transcriptional enhancer located 4.5 kb 3′ to the Cα gene segment. The TCR α enhancer contains four nuclear protein binding sites called Tα1–Tα4. In this report we describe the identification and characterization of a novel human cDNA, hGATA-3 that binds to the Tα3 element of the human TCR α enhancer. hGATA-3 contains a zinc finger domain that is highly related to the DNA-binding domain of the erythroid-specific transcription factor, GATA-1, and binds to a region of Tα3 that contains a consensus GATA binding site (AGATAG). Northern blot analyses of hematopoietic cell lines demonstrate that hGATA-3 is expressed exclusively in T cells. Overexpression of hGATA-3 in HeLa cells or human B cells specifically activated transcription from a co-transfected reporter plasmid containing two copies of the Tα3 binding site located upstream of the minimal SV40 promoter. Taken together these results demonstrate that hGATA-3 is a novel lineage-specific hematopoietic transcription factor that appears to play an important role in regulating the T cell-specific expression of the TCR α gene.

Key words: T cell/T cell receptor/transcription factor

Introduction

Hematopoietic development is characterized by the temporally-regulated expression of distinct sets of tissue-specific genes in different cell lineages. The molecular mechanisms underlying such developmentally-regulated, tissue-specific gene expression have been the subject of intense scrutiny during the last decade. A number of hematopoietic lineage-specific genes are transcriptionally regulated by enhancer elements that contain binding sites for different combinations of ubiquitous and tissue-specific nuclear proteins. Thus far, however, relatively few lineage-specific hematopoietic transcription factors have been identified. Oct-2, a lymphoid-specific homeobox protein has been shown to play an important role in regulating immunoglobulin gene expression during B lymphocyte ontogeny (Ko *et al.*, 1988; Muller *et al.*, 1988; Scheidereit *et al.*, 1988; Staudt *et al.*, 1988). Recently, GATA-1 (previously called GF-1 and Eryf1) an erythroid-specific zinc finger protein has been shown to regulate the transcription of a number of erythroid-specific genes including the α, β and γ globin genes (Evans *et al.*, 1988). To date, no T cell-specific transcription factors have been cloned and characterized.

We have utilized the human T cell receptor (TCR) α gene as a model system to study lineage-specific transcriptional regulation in T cells. The TCR α gene is rearranged and expressed exclusively in TCR α/β+ T cells, and its expression is controlled by a 694 bp T cell-specific transcriptional enhancer located 4.5 kb 3′ to the Cα gene segment (Ho *et al.*, 1989; Winoto and Baltimore, 1989a). Previous studies have demonstrated that this enhancer contains at least four nuclear protein binding sites or enhansons called Tα1–Tα4 (Ho and Leiden, 1990) (Figure 1). Tα1 contains a consensus cAMP response element (CRE) (TGACGTCA) and binds a set of ubiquitously expressed CRE binding proteins (CREBs) (Gottschalk and Leiden, 1990). Tα2 binds at least two T cell-specific nuclear protein complexes; TCF-1, a 53–57 kd nuclear protein (Waterman and Jones, 1990), and a second complex that includes the ETS-1 proto-oncogene product (Ho *et al.*, 1990). The Tα3 and Tα4 nuclear protein binding sites are not required for enhancer activity in mature T cell tumor cells, but may play an important role in regulating TCR α gene expression during early thymocyte ontogeny (Ho *et al.*, 1989; Ho and Leiden, 1990). Although the proteins that interact with the Tα3 and Tα4 elements have not been identified, DNase I footprint analyses revealed that the Tα3 element contains a potential binding site (AGATAG) for the GATA-1 transcription factor (Ho *et al.*, 1989). Because previous studies demonstrated that GATA-1 is not expressed in T cells (data not shown and Tsai *et al.*, 1989) we reasoned that the Tα3 element might bind a human T cell nuclear protein related to GATA-1.

In the studies described in this report, we used a fragment encoding the DNA binding region of the murine GATA-1 transcription factor (Tsai *et al.*, 1989) as a low stringency hybridization probe to isolate a novel human cDNA which we call human GATA-3 (hGATA-3). hGATA-3 is expressed in T cells but not in B cells, erythroid cells, or HeLa cells and encodes a 443 amino acid polypeptide which contains two zinc fingers that are 92% identical to those of GATA-1. hGATA-3 binds specifically to the sequence AGATAG in the Tα3 element of the human TCR α enhancer. Overexpression of hGATA-3 in HeLa cells results in the transactivation of TCR α enhancer-dependent transcription. Taken together with previous studies of GATA-1 (Evans and Felsenfeld, 1989; Tsai *et al.*, 1989; Romeo *et al.*, 1990, Martin *et al.*, 1990), these results demonstrate that members of the GATA gene family encode hematopoietic lineage-

specific transcription factors. Based on our data, it appears that hGATA-3 plays an important role in regulating the development of the T cell lineage.

Results

Isolation and structural characterization of hGATA-3 cDNAs

Because the Tα3 element contains a consensus binding site (AGATAG) for the GATA-1 transcription factor (Figure 1), we hypothesized that GATA-1 or a GATA-1 family member might bind to this site. Northern blot analyses demonstrated the absence of GATA-1 mRNA in a variety of T cell tumor cell lines (data not shown, and Tsai *et al.*, 1989). We used, therefore, a cDNA fragment encompassing the zinc finger region of GATA-1 as a probe in low stringency hybridizations to a cDNA library prepared from the human T cell tumor line Jurkat. This approach allowed the isolation of cDNA clones encoding a novel but related protein that we have called hGATA-3. DNA sequence analyses (Figure 2B) demonstrated that the 2.3 kb hGATA-3 cDNA contains a single long open reading frame (ORF) of 1329 bp beginning with an initiation codon at nucleotide 124. This ORF encodes a 443 amino acid polypeptide (predicted mol. wt = 48 kd). Although there are several in-frame initiation codons (nucleotides 124, 230, 704 and 737), we favor the hypothesis that the first of these is used because it closely resembles the consensus initiation codon described by Kozak (1984). This hypothesis is supported by the finding that *in vitro* transcription and translation of the hGATA-3 cDNA in a rabbit reticulocyte lysate resulted in a protein of approximate mol. wt = 47 kd as analyzed by SDS−PAGE (Figure 5A). Northern blot analyses demonstrated a single human GATA-3 mRNA of ~3.0 kb (Figure 4). The 2.3 kb hGATA-3 cDNA shown in Figure 2 appears to be lacking a portion of the 3′ untranslated region of the mRNA, and consequently lacks a consensus polyadenylation signal and polyA tail.

hGATA-3 contains two Cx zinc fingers (reviewed in Evans and Hollenberg, 1988) that are 92% identical to those of hGATA-1 (Zon *et al.*, 1990) (Figure 3B). The spacing (29 amino acids) between the zinc fingers is identical in both proteins. In addition, the regions at the COOH-terminal base of each finger are highly related to each other and have also been highly conserved between hGATA-3 and hGATA-1 (22/29 and 23/29 amino acid identities respectively) (Figure 3B). In contrast, regions of the two proteins outside of the zinc finger domains share only 12% amimo acid sequence identity (Figure 3A). Of note, the hGATA-3 protein lacks obvious glutamine- and proline-rich regions that have been described to function as transcriptional activator domains in other DNA-binding proteins (reviewed in Mitchell and Tjian, 1989). The NH_2-terminus of the protein is somewhat acidic (8 of 54 residues, net charge = −7). However, it remains unclear whether this region of the molecule functions as a classical acidic transcriptional activator domain. No leucine zipper (Landschulz *et al.*, 1988) or helix−loop−helix (Murre *et al.*, 1989) dimerization domains are present in hGATA-3.

hGATA-3 is expressed in cells of the T lymphocyte lineage

GATA-1 is a tissue-specific transcription factor that is expressed exclusively in erythroid cells, megakaryocytes, mast cells and their common progenitors (Martin *et al.*,

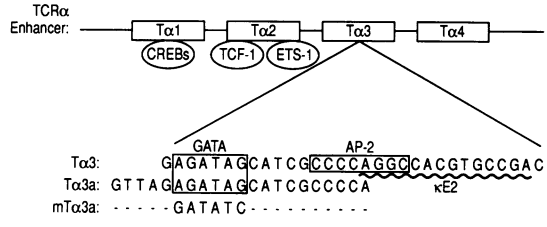
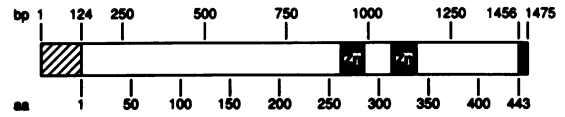


Fig. 1. Schematic representation of the human TCR α enhancer. The Tα1-Tα4 nuclear protein binding sites and their previously described cognate nuclear proteins are shown schematically. The nucleotide sequences of the Tα3, Tα3a, and mutant Tα3a (mTα3a) oligonucleotides used in these studies are shown at the bottom of the panel. The potential AP-2 and GATA (GATA) binding sites are boxed. The putative κE2 binding site is underlined.

A.



B.

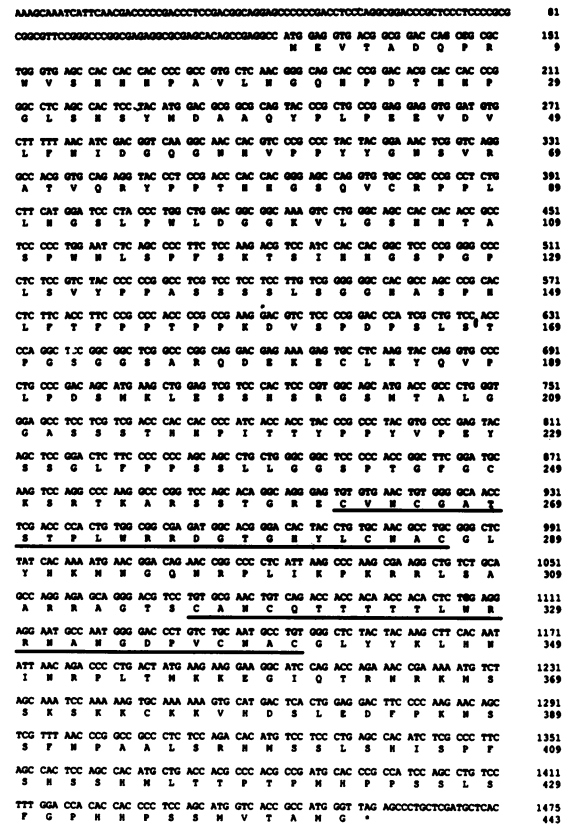


Fig. 2. Structure of the hGATA-3 cDNA and protein. The 2.3 kb hGATA-3 cDNA was isolated by low stringency hybridization with the GATA-1 zinc finger region probe as described in Materials and methods and subjected to dideoxy DNA sequence analysis. Nucleotides 1−1475, including the entire open reading frame, are shown. **A.** Schematic representation of the hGATA-3 cDNA. The 5′ untranslated region is cross-hatched, the open reading frame is unshaded and the 3′ untranslated region is solidly shaded. The position of the zinc fingers is shown (Zn). Base pairs (bp) and amino acids (aa) are numbered above and below the map respectively. **B.** Nucleotide and predicted amino acid sequence (EMBL accession no. X55037) of the hGATA-3 cDNA. The zinc fingers are underlined.

1990; Romeo *et al.*, 1990). To determine whether hGATA3 expression is restricted to specific hematopoietic lineages, we performed a Northern blot analysis using RNA derived from a series of T cell and non-T cell tumor cell lines (Figure 4). hGATA-3 was expressed as a single transcript of ~3.0 kb in all of the human T cell lines analyzed (Jurkat, HPB-ALL, Peer, and CEM). Murine EL4 T cells contained a single cross-hybridizing transcript of ~3.5 kb. Of note, the hGATA-3 gene was expressed in both TCR α/β^+ (Jurkat, HPB-ALL, and EL4) and TCR γ/δ^+ cells (Peer). In contrast, no expression was observed in two Epstein–Barr virus (EBV)-transformed human B cells (JY and Clone 13), in the erythroid tumor cell line K562 which is known to express high levels of GATA-1 (Tsai *et al.*, 1989), or in HeLa cells. CEM is an immature T cell line that in the unstimulated state expresses TCR β but not TCR α mRNA. However, stimulation of CEM cells with PMA results in the induction of TCR α mRNA synthesis (Shackelford *et al.*, 1987). Interestingly, PMA treatment also produced a substantial increase in hGATA-3 mRNA levels [Figure 4, compared CEM and CEM + PMA)].

hGATA-3 binds to the AGATAG sequence in Tα3

To study the interaction of hGATA-3 with the Tα3 element of the human TCR α enhancer, hGATA-3 protein produced by *in vitro* transcription and translation in a rabbit reticulocyte lysate was used in electrophoretic mobility shift assays (EMSAs) with a radiolabeled Tα3a oligonucleotide probe (Figure 5). SDS–PAGE analysis of the *in vitro* translated hGATA-3 product revealed a single predominant band of ~47 kd which was not seen in control translations performed without exogenous RNA (Figure 5A). As a control, human CREB RNA was also translated, and produced a single band of ~38 kd in agreement with previously published results (Hoeffler *et al.*, 1988). EMSAs performed with a radiolabeled Tα3a oligonucleotide probe and lysates from *in vitro* translation reactions programmed with either no RNA or with human CREB RNA produced a single band of altered mobility which presumably represented an AGATAG binding protein present in the reticulocyte lysate itself. This band appeared to bind specifically to the AGATAG sequence of the Tα3a probe because the binding was competed by excess unlabeled Tα3a competitor oligonucleotide but not by equivalent amounts of an unlabeled mutant Tα3a (mTα3a) oligonucleotide containing 6 nucleotide substitutions within the AGATAG sequence (Figure 5B).

In addition to the band seen with the control *in vitro* translation reactions, lysates containing *in vitro* transcribed and translated hGATA-3 produced a second band of altered mobility with the Tα3a probe (Figure 5B). This band which consistently migrated more slowly than the band seen with control translation reactions was not due simply to the inclusion of RNA in the translation reaction, because it was not seen when a variety of other transcription factor RNAs including CREB were *in vitro* translated and used in EMSAs with the hGATA-3 probe (Figure 5B). This second band represented specific binding of hGATA-3 to the AGATAG sequence in the Tα3a probe because the binding was competed by excess unlabeled Tα3a oligonucleotide but not by the mutant Tα3a oligonucleotide (mTα3a) (Figure 1) containing 6 nucleotide substitutions within the AGATAG sequence, or by the unrelated Tα1 oligonucleotide competitor (Figure 5B). Similarly, this band was not observed in binding reactions using hGATA-3 protein and a radiolabeled mTα3a

A.

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hGATA-3  MEVTADQPRWVSHHHPAVLNGQHPDTHHPGLSHSYMDAAQYPLPEEVDVLFNIDGQGNHVPPYYGNSVRA   70
hGATA-1  ------------------------------------------------------------MEFP LGSLG   10
NF-E1c     ST                    S   T G T   PT    A              P               70

hGATA-3  TVQRYPPTHHGSQVCRPPLLHGSLPWLDGGKVLGSHHTASPWNLSPFSKTSIHHGSPGPLSVYPPASSSS  140
hGATA-1   SEPL QFVDPAL SST ESGVFF SGPE LDAAASS  PSTATAAAAALAYYRDAEAYRHSPVFQVYPL   80
NF-E1c       TA                    S A S   S                S              T  140

hGATA-3  LSGGHASPHLFTFPPTPPKDVSPDPSLSTPGSGGSARQDEKECLKYQVPLPDSMKLESSHSRGSMTALGG  210
hGATA-1   NCMEGI GGSPYAGWAYGKTGLY ASTVCPTREDSPPQAV D DGKGSTSFLET KTERLSPDLLT  P  150
NF-E1c     A S                   I     T  T       I    S A T         S  AS     210

hGATA-3  A-SSSTHHPITTYPPYVPEYSSGLFPPSSLLGGSPTGFGCKSRPK--ARSST-GRECVNCGATSTPLWRR  277
hGATA-1   LP  LPV NSA GG-- DF   TF S TGSPLN AAYSSP L GTLPLPPCE A          A       217
NF-E1c     T  A                                              E                   278

hGATA-3  DGTGHYLCNACGLYHKMNGQNRPLIKPKRRLSAARRAGTSCANCQTTTTTLWRRNANGDPVCNACGLYYK  347
hGATA-1   R                          R  K  IVSK    Q T              S           287
NF-E1c                                                                           348

hGATA-3  LHNINRPLTMKKEGIQTRNRKMSSKSKKCKKVHDSLEDFPKNSSFNPAALSRHMSSLSHISPFSHSSHML  417
hGATA-1    QV      R D        A G G  KRGSSLGGTGAAEGPAGGFMVVAGGSG GNCGEVA GLTLGP  357
NF-E1c                                    N      S              I               418

hGATA-3  TTPTPMHPPSSLSFGPHHPSSMVTAMG                                            443
hGATA-1  PGTAHLYQGLGPVVLSGPV HLMPFP PLLGSPTGSFPTGPMPPTTSTTVVAPLSS               413
NF-E1c                                                                           444
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B.

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hGATA-3  CVNCGATSTPLWRRDGTGHYLCNAC
hGATA-1  CVNCGATATPLWRRDRTGHYLCNAC

hGATA-3  CANCQTTTTTLWRRNANGDPVCNAC
hGATA-1  CTNCQTTTTTLWRRNASGDPVCNAC
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Fig. 3. Sequence similarities between hGATA-3, hGATA-1 and NF-E1c. **A.** Overall amino acid sequence comparison. The hGATA-3, hGATA-1 (Zon *et al.*, 1990), and NF-E1c (Yamamoto *et al.*, 1990) amino acid sequences were aligned with the AAlign program of DNAstar software (Madison, WI). Gaps (–) were introduced to produce optimal alignment. Identical amino acids are shown as blank spaces in the hGATA-1 and NF-E1c sequences. **B.** Comparison of the zinc finger regions of hGATA-3 and hGATA-1. Amino acids conserved in all four zinc fingers are boxed.

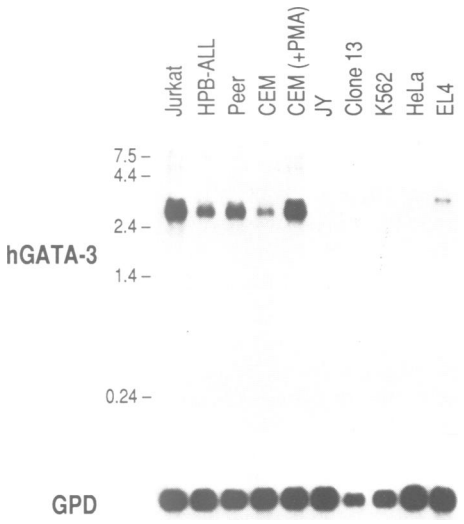

Fig. 4. Northern blot analysis of hGATA-3 expression in human and murine tumor cell lines. RNA from a variety of lymphoid and non-lymphoid tumor cell lines was subjected to Northern blot analysis with ^{32}P-labeled hGATA-3 (top panel) or glyceraldehyde-3-phosphate dehydrogenase (GPD; lower panels) cDNA probes. RNA size markers in kb are shown to the left of the autoradiogram.

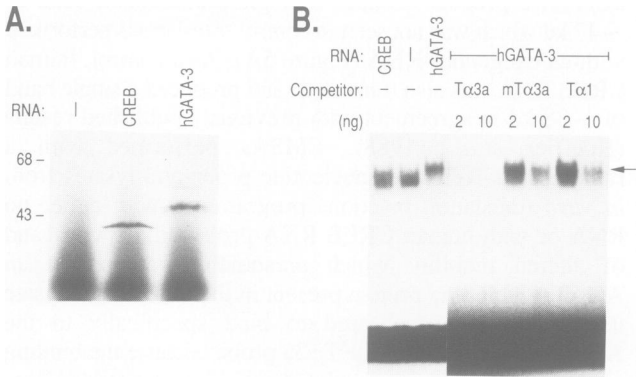

Fig. 5. Electrophoretic mobility shift assays using *in vitro* transcribed and translated hGATA-3. **A.** SDS–PAGE analysis of [^{35}S]methionine labeled *in vitro* translated materials. 20 μl of *in vitro* translation reactions containing no RNA (–), human CREB RNA (Hoeffler *et al.*, 1988)(CREB), or hGATA-3 RNA (hGATA-3) were analyzed by SDS–PAGE in a 9% denaturing gel. Size markers in kd are shown to the left of the autoradiogram. **B.** Electrophoretic mobility shift analysis of *in vitro* transcribed and translated proteins. *In vitro* translation reactions programmed with no RNA (–), human CREB RNA (CREB) or hGATA-3 RNA (hGATA-3) were used in EMSAs with a radiolabeled Tα3a probe (see Figure 1). Tα3a, mTα3a and control Tα1 (see Figure 1) cold competitor oligonucleotides were added in the amounts indicated to some of the binding reactions. The band of altered mobility corresponding to hGATA-3 binding is shown with an arrow. The competition experiments were subjected to a prolonged autoradiographic exposure to demonstrate the extent of competition by even small amounts of excess hGATA-3 competitor oligonucleotide.

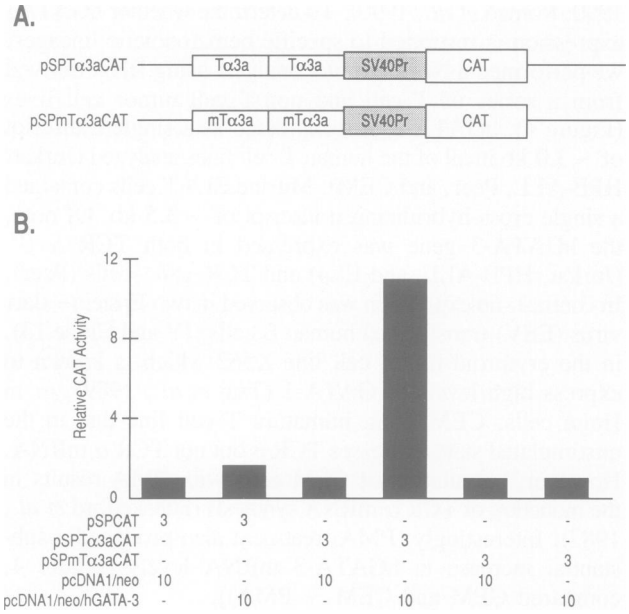

Fig. 6. Overexpression of hGATA-3 in HeLa cells activates transcription from co-transfected Tα3-containing reporter plasmids. **A.** Schematic representation of the pSPTα3aCAT and pSPmTα3aCAT reporter plasmids used in these studies. Two copies of the wild-type Tα3a (Tα3a) and mutant Tα3a (mTα3a) oligonucleotides were cloned into the *Sma*I site immediately 5′ to the minimal SV40 promoter (SV40 Pr) in the enhancerless pSPCAT reporter plasmid. **B.** Co-transfections of HeLa cells with the pSPCAT, pSPTα3aCAT or pSPmTα3aCAT reporter plasmids in combination with the pcDNA1/neo control plasmid or the pcDNA1/neo/hGATA-3 eukaryotic expression vector containing the hGATA-3 cDNA immediately downstream of the CMV promoter. The amount of DNA transfected in μg is shown in the bottom table. The data is expressed as CAT activities normalized to that produced by transfection of 3 μg of the pSPCAT reporter plasmid plus 10 μg of the pcDNA1/neo control plasmid (0.07% acetylation) following correction for differences in transfection efficiencies.

probe (data not shown). Although the hGATA-3-specific band somewhat obscured the reticulocyte lysate band, both bands could be clearly seen in the competition experiments using non-specific or mTα3a competitors (Figure 5B). From these data, we conclude that hGATA-3 binds in a sequence-specific fashion to the Tα3a probe. However, it should be emphasized that we cannot presently distinguish whether hGATA-3 binds to DNA as a monomer, homodimer, or a heterodimer in conjunction with endogenous proteins present in the reticulocyte lysate.

hGATA-3 activates transcription from reporter constructs containing the Tα3a element

To understand the role of hGATA-3 in regulating TCR α transcription, hGATA-3 was overexpressed in HeLa cells which do not normally express this protein (see Figure 4). Overexpression of hGATA-3 resulted in a significant (11-fold) activation of transcription from cotransfected reporter plasmids containing two copies of the Tα3a oligonucleotide cloned 5′ of the minimal SV40 promoter and the bacterial chloramphenicol acetyl transferase (CAT) reporter gene (Figure 6). The Tα3a element rather than the whole Tα3 site was used in the CAT reporter constructs in order to avoid the potentially confounding transcriptional activities of the κE2 and AP-2 sites locted at the 3′ end of Tα3 (but deleted from Tα3a). The transcriptional activator function observed in these experiments required both hGATA-3 protein and the AGATAG sequence because no transcriptional activation was observed in control transfections containing the pcDNA1/neo eukaryotic expression vector without the hGATA-3 cDNA insert, or in transfections using a reporter construct with 6 nucleotide substitutions in the AGATAG sequence of the Tα3a oligonucleotide (Figure 6). In additional experiments, overexpression of hGATA-3 in HeLa cells also activated transcription from a CAT reporter plasmid containing a single copy of the intact human TCR α enhancer by five-fold as compared to transfections utilizing the pCDNA1/neo control plasmid alone (data not shown). Moreover, overexpression of hGATA-3 in clone 13 EBV-transformed B cells also produced a 10-fold activation of transcription from a reporter plasmid containing two copies of the Tα3a oligonucleotide 5′ of the minimal SV40 promoter and CAT gene (data not shown).

Discussion

In this report we have described the isolation and functional characterization of a novel T cell transcription factor, hGATA-3. hGATA-3 contains two C*x* zinc fingers that are highly related to a similar domain in the erythroid-specific factor, GATA-1 and binds to a consensus GATA binding site (AGATAG) present in the Tα3 element of the human TCR α transcriptional enhancer. Overexpression of hGATA-3 in HeLa or clone 13 B cells specifically activated transcription from Tα3-containing reporter plasmids as well as from the intact TCR α enhancer. Thus, hGATA-3 is a hematopoietic lineage-specific transcription factor which functions, at least in part, to regulate TCR α gene expres-

sion in T cells. hGATA-3 belongs to a family of transcription factors which share highly related Cx zinc finger DNA binding domains, and bind to similar, if not identical, DNA sequences (WGATAR) in the transcriptional regulatory regions of a number of genes. The importance of this family of transcription factors is underscored by the recent identification of GATA-like proteins in fungi (Kudla *et al.*, 1990), chickens (Yamamoto *et al.*, 1990), and mammals (Evans and Felsenfeld, 1989; Tsai *et al.*, 1989). In vertebrates, some GATA family members (e.g. GATA-1 and GATA-3) appear to be lineage-specific transcriptional regulatory proteins (Martin *et al.*, 1990; Romeo *et al.*, 1990), while others (e.g. NF-E1b in the chicken, also called cGATA-2) appear to be more widely expressed (Yamamoto *et al.*, 1990). Similar differences in tissue distribution have been described among members of other families of transcription factors including the Octamer (Landolfi *et al.*, 1986; Singh *et al.*, 1986; Sive and Roeder, 1986; Staudt *et al.*, 1986) and ETS proteins (Bhat *et al.*, 1987). While this work was in progress, three GATA cDNAs isolated from a chicken reticulocyte library were described (Yamamoto *et al.*, 1990). One of these (NF-E1c) showed striking sequence similarity to hGATA-3 (Figure 3A) and was expressed preferentially in chicken T cells. Although the function of NF-E1c remains unknown, it is tempting to speculate that it represents the evolutionarily conserved homolog of hGATA-3, and will therefore play an important role in regulating TCR α gene expression in chicken T cells.

hGATA-1 and hGATA-3 share highly related zinc finger domains and bind to similar or identical sequences in the transcriptional regulatory regions of the globin and TCR α genes. Thus, the lack of TCR α gene expression in erythroid cells, and globin gene expression in T cells is probably determined by additonal factors such as chromatin structure, and/or the binding of other transcriptional regulatory proteins to the TCR α and globin enhancers. Nevertheless, the finding that the two proteins display only a very limited amount of amino acid sequence identity outside of their zinc finger domains, when taken together with the fact that they are expressed in a tissue-specific fashion, suggests that they may utilize distinct molecular mechanisms to regulate lineage-specific transcription. Whether hGATA-3 binds to DNA as a monomer or multimer, and which domains are required for putative protein–protein interactions and transcriptional activator functions remain unknown.

Previous studies have shown that the human TCR α enhancer is composed of multiple partially redundant nuclear protein binding sites or enhansons. At least three distinct T cell-specific protein complexes have now been shown to bind to the TCR α gene enhancer. In addition to hGATA-3 which binds to Tα3, TCF-1 (Waterman and Jones, 1990) and an ETS-1-containing complex have both been reported to bind to the Tα2 element (Ho *et al.*, 1990). The T cell-specific expression of these binding activities is likely to be responsible for the previously reported T cell specificity of TCR α enhancer activity (Ho *et al.*, 1989). However, the precise role of each of these factors in controlling the developmental regulation of TCR α gene expression will await ongoing studies designed to elucidate their patterns of expression and activities at different stages of fetal thymic development. It will be particularly interesting to determine the role of the Tα3 element in regulating TCR α gene expression during the early stages of thymocyte development. In addition to regulating TCR α gene expression, hGATA-3 might play a role in controlling the expression of other T cell-specific genes. In this regard, it is noteworthy that the δE4 site of the human TCR δ transcriptional enhancer contains two copies of a consensus hGATA-3 binding site (TGATAA) (Redondo *et al.*, 1990). None of the TCR α enhancer binding proteins described to date, including hGATA-3, are expressed exclusively in TCR α/β^+ cells. Therefore, the molecular mechanisms that restrict human TCR α gene expression to this cell lineage remain unknown. Transcriptional silencer elements such as those described in the murine TCR α locus (Winoto and Baltimore, 1989b) and/or additional lineage-restricted transcriptional activators may each play an important role.

Previous experiments have suggested that some tissue-specific transcription factors may be required for the differentiation and proliferation of specific cell lineages. Thus, for example, the Pit-1 gene is necessary for the development of somatoptrophs, lactotrophs, and thyrotrophs in the murine anterior pituitary, and mutations in this gene result in dwarf mice lacking these cell lineages (Li *et al.*, 1990). Similarly, it has recently been demonstrated that targeted mutations of mGATA-1 specifically block the development of the erythroid lineage in chimeric mice (Pevny *et al.*, 1990). Thus, it is tempting to speculate that GATA-3 may play a similarly important role in the proliferation and development of the T cell lineage. In this regard, it will be important to define the role of hGATA-3 in regulating the expression of other T cell genes, and to determine whether mutations in the hGATA-3 gene lead to immunodeficiencies in mice or men.

Materials and methods

Cell lines

Human T cell tumor lines Jurkat, Peer, CEM, and HPB-ALL as well as the EBV-transformed B cell lines, JY and Clone 13, and K562 human chronic myelogenous leukemia cells were grown in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FCS) and penicillin/streptomycin (Gibco) as described previously (Ho *et al.*, 1989). HeLa cells were grown in DMEM supplemented with 10% FCS and penicillin/streptomycin (Gibco).

Plasmids

The pSPCAT reporter plasmid containing the minimal SV40 promoter immediately 5′ to the bacterial chloramphenicol acetyl transferase (CAT) gene has been described previously (Leung and Nabel, 1988). The pSPTα3aCAT and pSPmTα3aCAT plasmids were made by cloning two copies of the Tα3a (5′-GTTAGAGATAGCATCGCCCCA-3′) or mTα3a (5′-GTTAGGATATCCATCGCCCCA-3′) oligonucleotides into the *Sma*I site immediately 5′ of the minimal SV40 promoter in pSPCAT (see Figure 6A). All oligonucleotides were synthesized on an Applied Biosystems model 380B DNA synthesizer. The identity of these constructs was confirmed by dideoxy DNA sequence analysis. The pcDNA1/neo/hGATA-3 expression vector was constructed by cloning the hGATA-3 cDNA (see Figure 1B) into the *Not*I site of the commercially available pcDNA1/neo eukaryotic expression vector (InVitrogen, San Diego, CA, USA).

Isolation and characterization of hGATA-3 cDNAs

A λgt11 cDNA library prepared from Jurkat poly(A)$^+$ RNA using random hexanucleotide primers (Ho *et al.*, 1990) was screened by low stringency hybridization to a 471 bp zinc finger region probe from the murine GATA-1 cDNA (bp 597–1068)(Tsai *et al.*, 1989). Hybridizations were carried out for 24 h at 50°C in 6 × SSC/0.1% SDS/100 μg/ml denatured salmon sperm DNA/5% dextran sulfate plus 2 × 10^6 c.p.m./ml of ^{32}P-labeled probe DNA prepared by random hexanucleotide priming (Specific activity > 10^9 c.p.m./μg). Filters were washed twice with 2 × SSC (1 × SSC = 0.3 M NaCl, 0.03 M Na citrate)/0.1% SDS for 15 min at room temperature

followed by a single 30 min wash in 2 × SSC/0.1% SDS at 42°C. Filters were exposed for autoradiography for 24 h at −70°C using intensifying screens. Positive plaques were purified to homogeneity by sequential screening using the same hybridization and washing conditions. cDNA inserts were cloned into the *Eco*RI site of pUC13 and subjected to dideoxy DNA sequence analysis as described previously (Ho *et al.*, 1989).

Northern blot analyses

RNA was prepared from tumor cell lines using the lithium chloride−urea method (Auffray and Rougeon, 1980). 1 μg of poly(A)$^+$ from each cell line was subjected to Northern blot analysis as described previously (Leiden *et al.*, 1989). Blots were hybridized with ^{32}P-labeled hGATA-3 and glyceraldehyde-3-phosphate dehydrogenase (GPD) (Dugaiczyk *et al.*, 1983) cDNA probes.

In vitro transcription/translation and electrophoretic mobility shift assays

In vitro transcription reactions were carried out using a commercially available kit (InVitrogen) according to the manufacturer's instructions. *In vitro* translation reactions were performed using a commercially available rabbit reticulocyte system (Promega, Madison, WI, USA) according to the manufacturer's instructions. For electrophoretic mobility shift assays, 2.5 μl of *in vitro* translated extract was mixed with 20 000 c.p.m. of ^{32}P-labeled oligonucleotide probe and 100 ng of poly dI:dC in a total volume of 15 μl of 1 × binding buffer (10 mM HEPES, pH = 7.8, 50 mM potassium glutamate, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 5% glycerol) and incubated at 4°C for 30 min. DNA−protein complexes were fractionated by electrophoresis at 200 V for 2 h at 4°C in non-denaturing 5% polyacrylamide gels prepared and run in 0.25 × TBE (0.22 M Tris−borate, 0.0005 M EDTA). Gels were dried and subjected to autoradiography for 4−24 h at −70°C.

Transfections and CAT assays

Clone 13 B cells were transfected by a modification of the DEAE−dextran method as described previously (Ho *et al.*, 1989). HeLa cells were transfected using calcium-phosphate (Gottschalk and Leiden, 1990). To control for differences in transfection efficiencies, each transfection also contained 2 μg of the pRSVβgal plasmid (Ho and Leiden, 1990). CAT and β-galactosidase activities were determined as described previously (Ho and Leiden, 1990) using cell extracts prepared 48 h after transfection.

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