GATA-3 Has Dual Regulatory Functions in Human Interleukin-5 Transcription*

Received for publication, August 15, 2001, and in revised form, September 11, 2001
Published, JBC Papers in Press, September 28, 2001, DOI 10.1074/jbc.M107836200

Gretchen T. F. Schwenger‡§, Régis Fournier, Chee Choy Kok‡, Viatcheslav A. Mordvinov, Deborah Yeoman‡, and Colin J. Sanderson‡

From the 2Department of Molecular Immunology, Curtin University of Technology, and the Western Australian Institute of Medical Research Level 5, Medical Research Foundation Building, Rear 50 Murray Street, Perth 6000, Western Australia, .toList Institute Francais des Boissons de la Brasserie et de la Malterie, Laboratoire de Biologie Moleculaire, 7, rue du Bois de la Champelle, F-54500 Vandoeuvre, France, and the Universite Libre de Bruxelles, Faculte de Medecine, Laboratoire d’Immunologie Experimentale, CP615, 808 route de Lennik, B1070 Brussels, Belgium

Interleukin-5 (IL-5) is a T-cell cytokine involved in Type 2 diseases and is commonly described as being coordinately regulated with other Type 2 cytokines, such as IL-4 and IL-13. Considering the unique control of eosinophilia by IL-5, such coordinate regulation would be surprising. In fact, the biological specificity of eosinophilia and its control by IL-5 suggests a unique and independent control of IL-5 regulation. In this report we show the binding of GATA-3 to three sites in the human IL-5 promoter in the human T-cell line PER117. The previously identified -70 site and another site at position -152 are shown to positively regulate IL-5 transcription. More importantly, the site located at -400 acts as a powerful repressor of IL-5 transcription with mutagenesis of this site allowing a high level expression of IL-5 without the activation of other factors normally required for IL-5 expression. Whereas GATA-3 has been proposed to be involved in the regulation of the IL-4/IL-5/IL-13 locus, we show here that it has another function in controlling IL-5 transcription that supports the observed unique biological function of this cytokine.

Eosinophilia is closely and uniquely controlled by the production of interleukin-5 (IL-5), primarily by activated T-cells. It is observed in a restricted pattern of disease, classically helminth infections and allergy. These fluctuations in eosinophil numbers can occur with little if any change in other leukocytes and are often not always associated with increased levels of IgE antibody (reviewed in Refs. 1 and 2). Taken together, the restricted and independent production of eosinophils suggests a restricted and independent control of IL-5 production.

However, IL-5 is commonly described as being coordinately expressed with other members of the locus (IL-4 and IL-13), especially IL-4. Such coordinate regulation is not consistent with the biology of eosinophilia. To objectively investigate the regulation of IL-5 expression, this assumption needs to be challenged. Two reports describe IL-5 and IL-4 as being predominantly produced by different human peripheral T-cells, indicating independent regulation of IL-4 and IL-5 (3, 4). In fact, the paper by Jung and colleagues (3) describe IL-4 and IL-5 as being “rarely co-expressed by human T-cells” and goes on to report that it is cloned T-cells that display the anomaly of co-expression of these cytokines. Thus, even in situations where both IL-4 and IL-5 are co-expressed in an individual in a Type 2 disease, it appears that they are expressed in different T-cell populations. How is this general tendency to express Type 2 cytokines in these diseases regulated at the gene level when they are apparently not coordinately expressed at the cell level?

GATA-3 is predominantly expressed by T-cells (5, 6) and is critical for thymocyte development (7) and for the regulation of effector T-cells (8). Ectopic expression of GATA-3 in developing Type 1 cells gives rise to Type 2 cytokine production (8–10) as well as to Type 1 cytokine inhibition (9, 10). Ectopic expression of GATA-3 has similarly been shown to induce Th2 cytokine expression in committed Type 1 cells (11). T-cell-specific expression of a dominant-negative mutant in mice decreased Type 2 cytokine expression, thus reducing eosinophil and IgE production (12). Conversely, the expression of GATA-3 in T-cells in transgenic mice induced Type 2 commitment with increased capacity for Type 2 cytokine production (13). These experiments are consistent with a role for GATA-3 in the regulation of the IL-4/IL-5/IL-13 locus (14), but they do not provide an explanation for the unique characteristics of IL-5 expression. The promoters of IL-4, IL-5, and IL-13 have little in common and may provide the basis for independent expression.

Whereas GATA has not been shown to have a direct effect on the transcription of the IL-4 promoter (12, 15), it does interact with a site (−70) in the IL-5 promoter. This is necessary but not sufficient for IL-5 expression (16, 17) and may be an important factor in the differential expression of IL-4 and IL-5. Another important difference lies in the conserved lymphokine element 0 (CLE0), which shows low conservation between these two cytokine genes (18). In the IL-5 promoter (−42 to −56), it plays a major role as the on/off switch for IL-5 transcription. It binds activator protein 1 (AP-1) members JunD and Fos-related antigen 2 as well as Oct-1 and Oct-2 factors and is critical for IL-5 expression (19, 20). Additional activation signals for IL-5 come from the nuclear factor of activated T-cells site at position −110 (21, 22). We have recently also identified two repressor elements, HPRE1-IL5 and HPRE2-IL5 in the proximal and distal IL-5 promoters, respectively. Because of the apparent impor-
GATA-3 Has Dual Functions in IL-5 Regulation

GATA-3 in the regulation of the locus, we have undertaken a detailed analysis of its role in transcription. Similar to the reports for IL-4 (23), we find no evidence for a transcriptional role in IL-13. However, we have identified two further GATA binding sites in the IL-5 promoter. One of these binding sites (at \(-70\)) acts like the \(-70\) site. Mutagenesis significantly lowers IL-5 expression, indicating that the binding of GATA-3 is necessary for expression. Remarkably, the other site at \(-400\) acts as a powerful repressor. Mutagenesis allows high level expression without the activation of other transcription factors normally required for IL-5 expression. This suggests that although GATA-3 appears to have some overriding control of the locus, it has unique transcriptional effects on IL-5 and the subsequent eosinophilia.

EXPERIMENTAL PROCEDURES

Cell Lines and Plasmids—PER117 cells were grown in RPMI 1640 medium supplemented with 7.5% fetal calf serum (Trace Scientific, Victoria, Australia), 100 \(\mu\)M minimum Eagle’s medium non-essential amino acid solution (Life Technologies, Inc.), 1 mM sodium pyruvate.

Fig. 1. Sequences protected by PER117 nuclear proteins in the \(-506\) to \(-184\) fragment of the hIL-5 promoter. DNase I protection experiments were carried out with double strand probes labeled either on the 5’ extremity of the coding strand (panel A, left picture) or of the non-coding strand (panel A, right picture). Prior to digestion, the probes were incubated with nuclear proteins extracted from PER117 cells, which were either unstimulated (lanes 2, 4, 7, and 9) or 16-h PMA/cAMP stimulated (lanes 5, 8, and 10). The digestion of the probe alone was performed either with no protein and 0.01 unit of DNase I or with 50 or 100 \(\mu\)g of protein and 1.0 unit of DNase I. B, positioning of the protected sequences BR6, BR7, and PRE2 was achieved by enzymatic sequencing of the probe. The sequences protected from digestion by the DNase I are underlined.

Fig. 1A. Sequences protected by PER117 nuclear proteins in the \(-506\) to \(-184\) fragment of the hIL-5 promoter. DNase I protection experiments were carried out with double strand probes labeled either on the 5’ extremity of the coding strand (panel A, left picture) or of the non-coding strand (panel A, right picture). Prior to digestion, the probes were incubated with nuclear proteins extracted from PER117 cells, which were either unstimulated (lanes 2, 4, 7, and 9) or 16-h PMA/cAMP stimulated (lanes 5, 8, and 10). The digestion of the probe alone was performed either with no protein and 0.01 unit of DNase I or with 50 or 100 \(\mu\)g of protein and 1.0 unit of DNase I. B, positioning of the protected sequences BR6, BR7, and PRE2 was achieved by enzymatic sequencing of the probe. The sequences protected from digestion by the DNase I are underlined.
GATA-3 Has Dual Functions in IL-5 Regulation

(From Life Technologies, Inc.), 2 mM L-glutamine (Sigma), 75 μM monothioglycerol (Sigma), and 10 mM Hepes, pH 7.3 (Life Technologies, Inc.).

The pCR2-1hIL-5p plasmid was used to isolate a 533-bp hIL-5 promoter fragment. The fragment BamHI (−509) to EcoRI (44) from pCR2-1hIL-5p was then subcloned in the BamHI/EcoRI site of the pALTER vector (Promega), producing plasmid pALTER-hIL-5. Site-directed mutagenesis was performed using the Altered Sites II in vitro mutagenesis system (Promega) with the oligonucleotides listed in Table 1. Mutations were confirmed by DNA sequencing.

Following confirmation of mutations, the eight mutant and single wild-type hIL-5 fragments were isolated from the respective pALTER-hIL-5 and pALTER-hIL-5mut plasmids by BamHI/EcoRI digest and ligated into the BamHI/EcoRI sites of pSP72 (Promega) creating pSP-hIL-5 and pSP-hIL-5mut constructs. The pSP-hIL-5 and pSP-hIL-5mut constructs were subsequently digested with XhoI and BglII, isolated, and ligated into NheI/BglII-digested pGL3-Basic (Promega). The resulting constructs were called hIL-5p, hIL-5-400c, hIL-5-400cnc, hIL-5-400cnc, hIL-5-128, hIL-5-70c, hIL-5-70nc, and hIL-5-70nc and were used for transfection experiments.

DNase I Protection Assays and Electrophoretic Mobility Shift Assays—Nuclear extracts used in electrophoretic mobility shift assays (EMSA) and DNase I protection assays were prepared as described previously (24). The probe used in DNase I protection assays was obtained by subcloning a DNA fragment covering the promoter sequence from positions −509 to +184 in the Smal site of the vector pSP72 (Promega). The fragment was obtained from the construct pCR2-1hIL-5 (24). The fragment was excised with either SfiI/KpnI to allow selective 5′ labeling of the coding strand or by EcoRI/PstI for the selective 5′ labeling of the non-coding strand. The DNase I protection assays, the labeling and purification of the probes, and the localization of the protected sequences on the coding and non-coding strands were performed as described previously (24). 50 or 100 μg of protein from either unstimulated or 16-h phorbol 12-myristate 13-acetate (PMA)/
dibutyryl cyclic adenosine monophosphate (cAMP)-stimulated PER117 cells were used to probe the fragment. Naked DNA was incubated with 0.01 unit of DNase I (Life Technologies, Inc.) or 1.0 unit when incubated with proteins.

EMSA were performed as described previously (24). 3 μg of protein were pre-incubated 20 min on ice in 60 mM KCl, 8 mM MgCl2, 12 mM Hepes, pH 7.9, 0.1 mM EDTA, 1 mM dithiothreitol, 12% glycerol in a final volume of 18 μl prior to addition of 25 fmol of 5′-labeled double-stranded probe. For competition experiments, double-stranded competitor oligonucleotides were added before the probe and incubated for a subsequent 20 min. For supershift experiments, the antisera were added before the probe and incubated for 1 h.

Polyclonal antibodies against AP1, Oct-1, Oct-2, C/EBPβ, C/EBPε used in the supershift experiment were purchased from Santa Cruz Biotechnology, Inc. The anti-GATA-3 and GATA-4 antibodies were a generous gift from Dr. Tetsuya Yamagata (17). Oligonucleotides used in EMSA are listed in Table 1. Probe preparation, binding reactions, and polyacrylamide gel electrophoresis were performed as described previously (25). EMSA pictures were generated by a PhosphorImager (Molecular Dynamics Inc.).

Transfection Protocol—All transfections were carried out in triplicate. 15 μg of DNA was electroporated at 960 μFd and 280 V into 107 PER117 cells in 400 μl of growth medium using the Bio-Rad gene pulser. After electroporation, the cells were incubated at 37 °C for a period of 6 h before activation. For T-cell activation, PMA (Sigma), cAMP (Sigma), and anti-Leu-CD28 (Becton Dickinson Immunocytochemistry Systems) were used at concentrations of 10 ng/ml, 1 μM, and 0.2 μg/ml, respectively. After an additional incubation period of 16 h, the

FIG. 2. Map of the hIL-5 promoter indicating predicted GATA binding sites. Predicted GATA binding sites are shown in relation to the CLE0 and PRE2 sites. Positions of the sites are given, and orientations of the homologous GATA sequences are indicated with an arrow.
cells were harvested and resuspended in 100 μl of reaction buffer containing 50 mM Tris-HCl, pH 7.8, 15 mM MgSO₄, 33.3 mM dithiothreitol, 0.1 mM EDTA, 250 μM LiCoA (Sigma), 500 μM sodium-luciferin (Molecular Probes Inc.) and 0.5% Triton X-100. Luciferase activity was measured in a Victor 1420 multilabel reader (Wallac, Turku, Finland). Therefore, this promoter was analyzed using TFSearch (27). As shown on Fig. 1, BR7 contains the consensus binding sites for the transcription factors Oct-1 and C/EBP. BR6 sequence analysis through the same program revealed two potential binding sites for a member of the GATA transcription factor family, each positioned in reverse orientation from the other and therefore found to be part of a GATA palindromic site (Fig. 1B). The sites are not only remarkable by the fact that they are palindromic, but also because they are non-canonical (e.g. AGATTA instead of the AGATATA). In addition, the search revealed matches with consensus binding sites of Oct, AP-1, and C/EBP transcription factors in BR6.

GATA has been shown to play no significant role in hIL-4 transcription (23), and no information was available for hIL-13. Therefore, this promoter was analyzed using TFSearch (27) along with the hIL-5 (−509 to +44) promoter for other potent-
GATA-3 Has Dual Functions in IL-5 Regulation

Fig. 5. Biological Activity of −70, −152, and −400 GATA sites in hIL-5. Transfection of mutant hIL-5 promoter reporter constructs was carried out in PER117 cells. Luciferase activity was determined after 16 h with and without 16 h of stimulation with 10 μg/ml PMA and 1 mM cAMP or 10 μg/ml PMA and 0.2 μg/ml anti-CD28. A, transfection of wild-type (hIL5p) and −70 GATA mutants on the coding hIL5−70c, non-coding hIL5−70nc, or both hIL5−70cnc strands with PMA/cAMP stimulation. B, transfection of wild-type (hIL5p) and −70 GATA mutants on the coding hIL5−70c, non-coding hIL5−70nc, or both hIL5−70cnc strands with PMA/CD28 stimulation. C, transfection of wild-type (hIL5p)−128 GATA mutant and −152 GATA mutant with PMA/cAMP stimulation. D, transfection of wild-type (hIL5p)−128 GATA mutant and −152 GATA mutant with PMA/CD28 stimulation. E, transfection of wild-type (hIL5p) and −400 GATA mutants on the coding hIL5−400c, non-coding hIL5−400nc, or both hIL5−400cnc strands with PMA/cAMP stimulation. F, transfection of wild-type (hIL5p) and −400 GATA mutants on the coding hIL5−400c, non-coding hIL5−400nc, or both hIL5−400cnc strands with PMA/CD28 stimulation.

Potential GATA binding sites. Four potential sites were identified in the first 900 bp of the hIL-13 promoter: 1) hIL13GATAa (−87 to −78), 2) hIL13GATAb (−385 to −377), 3) hIL13GATAc (−694 to −686), and 4) hIL13GATAd (−884 to −876). Of these sites, hIL13GATAa, hIL13GATAb, and hIL13GATAd bound proteins from unstimulated and stimulated PER117 cells, but none of these were identified by competition or supershift EMSA as GATA proteins.

Although the binding in the IL-5 promoter of GATA-3 protein at the site located at position −70 was well established in the mouse, it has never been clearly demonstrated in the human. Similar to the −400 GATA site, −70 GATA contains two sites...
arranged in repeated inverted orientation. In addition, the presence of an additional GATA binding site located further upstream has been reported a few years ago by Prieschl et al. (21). This site was described as being located between positions −177 and −80. A closer examination of this region showed that two potential sequences matching a consensus GATA binding site are present in this area. One was located at −128 and the other at −152. Both are in the same orientation and do not present any palindromic features (Fig. 2). DNaseI protection analysis of the proximal hIL-5 promoter identified the protection of the −152 but not the −128 site, and the −70 GATA site is also clearly protected (24).

GATA-3 Binds to the −400, −152, and −70 GATA Sites—EMSA analysis was performed in order to identify experimentally the proteins protecting the BR6 and BR7 regions. The analysis was made with nuclear extract prepared from unstimulated PMA/cAMP- or PMA/CD28-stimulated PER117 cells. With all three extracts, the banding patterns obtained were the same, and only the results obtained with the PMA/cAMP-stimulated extracts are shown in Fig. 3A. A complete absence of binding is observed with BR7 probe (data not shown), but the strong protection of this sequence especially on the non-coding strand strongly suggests that this element is of importance (Fig. 1A, lane 2). This is described under “Discussion.”

When using the −400/BR6 oligonucleotide, two complexes are obtained (Fig. 3A, lane 1). The two complexes are denominated −400L (low mobility complex) and −400H (high mobility complex) and are specific to the sequence, because they are completely competed by an excess of 50 times of unlabeled probe −400/BR6 but not the unrelated AP-2 oligonucleotide. The bands visible ahead of the described complexes are non-specific binding, because they are not removed by the unlabeled probe competition or not constantly present. They will not be further considered.

To identify the nature of the factors comprising complexes −400H and −400L, the competition with 50× excess unlabeled oligonucleotides containing the consensus binding sites of the factors described above (Fig. 3A) were performed. The GATA consensus site oligonucleotide inhibits the formation of both complexes, although the inhibition is only partial for −400L (lane 3). C/EBP consensus site completely inhibits the formation of the −400H complex and affects partially −400L formation (lane 5). The Oct, AP-1, and AP-2 sequences were unable to interfere with the formation of any of the complexes (lanes 4, 6, and 7).

The identification of the proteins binding on the BR6 sequence was achieved by supershift experiments utilizing specific antibodies against two members of the GATA transcription factor family, GATA-3 and -4, as well as antibodies raised against the factors C/EPBβ and C/EPBε and Oct-1 and -2. Anti-GATA-3 antibodies were able to inhibit both −400 complexes (Fig. 3A, lane 8), whereas anti-GATA-4 antibodies were unable to produce a shift. The other antibodies were unable to shift either of the −400 complexes (data not shown). These results clearly indicate that the complexes formed on the BR6 sequence contain GATA-3 proteins.

To further characterize the GATA binding ability of the two sites present at positions −399 and −393, EMSA experiments were performed with oligonucleotides containing the mutations of one, the other, or both GATA sites present in BR6 sequence. For convenience of identification, the GATA site located on the coding strand was named GATAc, and the GATA site located on the non-coding strand was called GATAnc. As shown on Fig. 3B, a disruption of the GATA-coding strand sequence (AGATTA to ACTTGA) does not impair the binding of GATA protein (lane 2), when the same mutation made on GATA non-coding strand prevents the binding of the probe (lane 3). The double mutant presents the same binding pattern as the GATA non-coding mutant (lane 4). Thus, only the site located on the negative strand of the palindromic sequence is able to bind GATA factors.

Three probes were designed spanning the −70, −128, and −152 GATA sites. The ability of these double-stranded oligonucleotides to bind proteins from PER117 cells was analyzed by EMSA. As shown on Fig. 4, A and B, respectively, both −70 GATA and −152 GATA are able to give rise to two complexes of similar mobility to −400H and −400L. In contrast, the −128 GATA site was unable to form complexes with PER117 nuclear extracts (data not shown). The use of antibodies against GATA proteins shows that the complexes, as described previously for −400 GATA, contain GATA-3 protein but not GATA-4 (Fig. 4, A and B). The high mobility complex obtained with −70 GATA probe was not altered by the anti-GATA antiserum.

Biological Activity of the −400, −152, and −70 GATA Elements—To assess the activity of the GATA elements in the
context of the 509-bp promoter, a study by site-directed mutagenesis was performed. The three −400 GATA site mutations described previously (m-400GATAnlc, m-400GATAc, and m-400GATAncc) for which the binding ability of GATA proteins was verified (Fig. 3B) were introduced into the hIL-5 509-bp promoter. Similar mutations were made in the −70 palindromic GATA site. The two single non-palindromic GATA sites at positions −128 and −152 were also mutated. All mutations were tested in EMSA and shown to abolish GATA binding to these sites (data not shown).

Mutant expression constructs were transfected into PER117 cells as described under “Experimental Procedures,” and luciferase activity was determined after 16-h stimulation with or without PMA and cAMP or PMA and anti-CD28. Fig. 5, A and B, show a dramatic reduction in hIL-5 promoter activity upon the mutation of the non-coding strand −70 GATA site. Expression was reduced to 7% of the wild-type level in PMA/cAMP-stimulated cells and 9% of the wild-type level in PMA/CD28-stimulated cells, whereas the mutation of the coding strand −70 GATA site only had a nominal effect on expression. Mutation of both sites together showed a similar result to the single mutation in the non-coding −70 GATA site. As expected, the mutation of the −128 GATA (Fig. 5, C and D) site did not result in any significant change in hIL-5 promoter activity. In contrast, the mutation of the −152 GATA site reduced expression to 18% of wild-type levels under PMA/cAMP stimulation (Fig. 5C) and 14% of wild-type levels under PMA/CD28 stimulation (Fig. 5D).

The most interesting results were obtained with the mutation of the −400 GATA site (Fig. 5, E and F). Individual mutation of the GATA sites on opposite strands had no effect on hIL-5 promoter expression. In contrast, mutation of both the coding and non-coding strands together resulted in a 2–3-fold increase of stimulated activity and, more importantly, up to a 250-fold increase in unstimulated activity of the hIL-5 promoter. This dramatic change increased constitutive expression to 1.54 ± 0.27 times the level of wild-type stimulated expression. The possible implications of this finding is described under “Discussion.”

**DISCUSSION**

We demonstrate here that the T-lymphocyte-specific transcription factor GATA-3 is directly involved in the regulation of the hIL-5 gene expression at three sites of the IL-5 promoter. The involvement of GATA-3 in the activation of IL-5 transcription was shown in the mouse (28), and another study showed that it has the same role in man (16). Using DNase I protection assays, we have shown that a sequence close to the repressor element PRE2 (29) is strongly protected when incubated with PER117 nuclear proteins, and that this sequence binds GATA-3. We used the human T-cell line, PER117, which expresses IL-5 and other cytokines in an inducible fashion. As in primary T-cells, there is no detectable constitutive expression of IL-5, but expression can be induced with PMA and further enhanced by cAMP, ionomycin, and anti-CD28 antibodies. Furthermore, the response of IL-5 to cyclosorpin A and dexamethasone is the same in PER117 cells as in primary human T-cells suggesting that PER117 cells express IL-5 in a physiological manner (26, 30).

GATA proteins can bind on their recognition sequence as dimers or monomers (12). The binding of GATA on the BR6 site generated two complexes, which may contain either a GATA heterodimer (−400L) or a GATA monomer (−400H). Similarly, the −70 GATA site formed two complexes. The palindromic nature of −70 GATA and −400 GATA sites is not sufficient to explain the heterodimer binding, because this higher mobility complex is also present on the single site of the −152 GATA probe.

The high mobility complex −400H is highly competed by an oligonucleotide-bearing consensus sequence of C/EBP binding sites but is not recognized by antisera against C/EBPα or anti-C/EBP β. The competition observed might be attributed to non-specific interactions, but it is not unreasonable to consider the possibility that a C/EBP factor binds on this site. Indeed, C/EBP factors are expressed in Jurkat T-lymphocytes as well as several cell types of the hematopoietic lineage (31) and can activate the transcription of a cytokine gene (IL-8) by interacting with nuclear factor-κB (32).

A striking difference among the binding patterns of the three sites is the complete lack of reactivity of −70 GATA higher mobility complex with the GATA antisera. This complex is not recognized by the C/EBP antibodies, even though it is completely removed by a 50× excess of an oligonucleotide bearing the consensus C/EBP binding site sequence. The nature of this complex remains unknown, but the high similarity of the electrophoretic characteristics of all the high mobility complexes generated on GATA probes tends to indicate that these complexes are related and specific for GATA sequences.

The −70 GATA site is particularly interesting in terms of its close position to the transcription start. Indeed, the interaction of GATA proteins with factors that are bound to the active CLE0 element is highly probable. This possibility is supported by the cooperative binding of GATA-3 with Ets factors in the myeloid Kasumi cell line (16), and it is very probable that GATA takes part in the multiprotein complex occurring on the CLE0 sequence after activation of the cells. In the same study, the existence of a cellular factor mediating the promoter activation by ionomycin was proposed, and the high mobility complex that we have obtained on the −70 GATA sequence could be related to this factor.

No complexes were obtained with the oligonucleotide containing the sequence BR7, which is surprising considering the intensity of the observed protection of this region. It is possible that the proximity of the PRE2 element to the BR6 allows them to interact, and the protection observed in the BR7 region may result from consequent DNA conformational changes.

The −152 GATA is a single site that is able to bind GATA-3 factors and has a positive effect on hIL-5 transcription. The footprinting pattern obtained in this region (24) reveals a strong protection whether DNase I protection assays were carried out with unstimulated or stimulated PER117 cells. The −128 site has a high homology to the consensus GATA sequence, yet it is not protected in DNase I protection assays (24) and does not bind GATA, indicating the importance of binding site environment.

The importance of tandem-inverted GATA binding sites has been described previously, in particular for GATA-1 (reviewed in Ref. 33). The promoters of several erythroid cell-specific genes have been shown to contain bipartite GATA binding sites, yet they bind a single molecule of GATA-1. This study shows that the binding of GATA-1 on a GATA palindromic site will be stronger than on a single site. In erythroid cells, the specific formation of hypersensitive sites in the human β-globin gene promoter is dependent on the presence of tandem-inverted GATA binding sites, which are specific for GATA-1 binding. The disruption of the GATA binding site abolished the formation of the hypersensitive sites (34). In EL-4 cells and a murine promoter, it was shown that the affinity of GATA-3 is greater for the site located on the negative strand (28). In acute T-cell leukemia-16 cells, GATA-4 also binds on the negative strand site (17). The results we have obtained show that, in the cell line PER 117, GATA-3 also binds only on the negative
strands at the −70 and −400 sites. Indeed, an oligonucleotide carrying a mutation of the negative strand site is unable to compete with the binding of GATA-3 protein on the −70 site in EMSA. The two GATA sites at position −400 are strictly identical in sequence but not in their ability to bind GATA-3 protein, since only the negative strand is able to do so.

Here we describe the −400 GATA site acting as a repressor of hIL-5 expression under stimulation in the human T-cell line PER117. However, an even more striking finding was the massive up-regulation of basal activity of the promoter when −400 GATA-binding was abolished. To our knowledge, such a function has not previously been reported for GATA-3. We propose a model whereby the constitutive binding of GATA-3 to its sites in the hIL-5 promoter is necessary for maintaining a specific DNA structure that is crucial for the regulation of the gene. We suggest that the binding of GATA-3 to the −400 site induces a conformational change that results in the protection of BR7, which itself does not bind proteins. This conformational change may bring together upstream regulators, such as the previously identified hPRE2 repressor element, and such as inhibit IL-5 expression. When GATA-3 does not bind to the −400 site because of mutation, the structure of the promoter is altered, and the gene expresses at a very high level apparently over because of mutation, the structure of the promoter is altered, and the gene expresses at a very high level apparently over-

Acknowledgment—We thank Professor T. Yamagata of the University of Tokyo for the generous gift of anti-GATA antisera.

REFERENCES

1. Sanderson, C. J. (1992) Blood 79, 3101–3109
2. Schwenger, G. T., Mordvinov, V. A., Fourrier, R., Czabotar, P., Peroni, S., and Sanderson, C. J. (2000) Academic Press, DOI:10.1006/rwcy.2000.0902
3. Jung, T., Schauer, U., Rieger, C., Wagner, K., Emsel, K., Neumann, C., and Arai, N. (1993) Eur. J. Immunol. 23, 2413–2416
4. Krug, N., Jung, T., Napp, U., Wagner, K., Schultz-Werninghaus, G., Heusser, C., Rieger, C. H., Schauer, U., and Fabel, H. (1998) Am. J. Respir. Crit. Care Med. 157, 754–760
5. Ho, I. C., Vorhees, P., Marin, N., Oakley, B. K., Tsai, S. F., Orkin, S. H., and Leiden, J. M. (1991) EMBO J. 10, 1187–1192
6. Ko, I. J., Yamanoto, M., Leonard, M. W., George, K. M., Ting, P., and Engel, J. D. (1991) Mol. Cell. Biol. 11, 2778–2784
7. Ting, C. N., Olson, M. C., Barton, K. P., and Leiden, J. M. (1986) Nature 324, 478–480
8. Zheng, W., and Flavell, R. A. (1997) Cell 89, 578–596
9. Ouyang, W., Ranganath, S. H., Weindel, K., Bhattarcharya, D., Murphy, T. L., Sha, W. C., and Murphy, K. M. (1998) Immunity 9, 745–755
10. Ferber, I. A., Lee, H. J., Zolin, F., Heath, V., Mui, A., Araí, N., and O’Garra, A. (1999) Clin. Immunol. 91, 134–144
11. Lee, H. J., Takemoto, N., Kurata, H., Kamogawa, Y., Miyatake, S., O’Garra, A., and Araí, N. (2000) J. Exp. Med. 192, 105–115
12. Zhang, D. H., Yang, L., Cohn, L., Parkyn, L., Homer, R., Ray, P., and Araí, N. (1999) Immunology 111, 473–482
13. Nawijn, M. C., Dinjgaan, G. M., Ferreira, R., Lambrecht, B. N., Karis, A., Graveweld, F., Savelkoul, H., and Hendriks, H. W. (2001) J. Immunol. 167, 724–732
14. Takemoto, N., Kamogawa, Y., Jun Lee, H., Kurata, H., Araí, K. I., O’Garra, A., and Araí, N. (2000) J. Immunol. 165, 6687–6691
15. Lee, H. J., O’Garra, A., Araí, K., and Araí, N. (1998) J. Immunol. 160, 2343–2352
16. Blumenthal, S. G., Aichele, G., Wirth, T., Czerwinski, A. P., Nordheim, A., and Dittmer, J. (1999) J. Biol. Chem. 274, 12900–12906
17. Yamagata, T., Nishida, J., Sakai, R., Tanaka, T., Honda, H., Hirano, N., Mano, H., Yazaki, Y., and Hirai, H. (1995) Mol. Cell. Biol. 15, 3830–3839
18. Masuda, E. S., Tokumitsu, H., Tsuibo, A., Sbloma, J., Hung, P., Araí, K., and Araí, N. (1995) Mol. Cell. Biol. 15, 7399–7407
19. Thomas, M. A., Mordvinov, V. A., and Sanderson, C. J. (1999) Eur. J. Biochem. 265, 300–307
20. Mori, A., Kamishima, O., Mikami, T., Hoshino, A., Ohmura, T., Miyazawa, K., Suko, M., and Okudaira, H. (1997) Int. Arch. Allergy Immunol. 113, 272–274
21. Prieschel, E. E., Goilleux-Gruart, V., Walker, C., Harrer, N. E., and Baumrukner, T. (1995) J. Immunol. 154, 6121–6139
22. De Boer, M. L., Mordvinov, V. A., Thomas, M., and Sanderson, C. J. (1999) Int. J. Biochem. Cell Biol. 31, 1221–1236
23. Ranganath, S., Ouyang, W., Bhattacharya, D., Sha, W. C., Grupe, A., Pelz, G., and Murphy, K. M. (1998) J. Immunol. 161, 3822–3826
24. Mordvinov, V. A., Schwenger, G. T., Fourrier, R., De Boer, M. L., Peroni, S. E., Singh, A. D., Karlen, S., Holland, J. W., and Sanderson, C. J. (1999) J. Allergy Clin. Immunol. 103, 1125–1135
25. Karlen, S., and Beard, P. (1993) J. Virology 67, 4296–4306
26. Mordvinov, V., Peroni, S., De Boer, M., Kees, U., and Sanderson, C. J. (1999) J. Immunol. Methods 228, 163–168
27. Heinemeyer, T., Wingender, E., Reuter, I., Herrnhaak, H., Kel, A. E., Kel, O. V., Ignatieva, E. V., Ananko, E. A., Podkolodnaya, O. A., Kulpakov, P. A., Podkolodny, N. L., and Kolchanov, A. A. (1996) Nucleic Acids Res. 24, 362–367
28. Siegel, M. D., Zhang, D. H., Ray, P., and Ray, A. (1995) J. Biol. Chem. 270, 24548–24553
29. Schwenger, G. T., Fourrier, R., Hall, L. M., Sanderson, C. J., and Mordvinov, V. A. (1999) J. Allergy Clin. Immunol. 104, 820–827
30. Kees, U. R., Peroni, S. E., Ranford, P. R., and Ford, J. (1994) J. Immunol. Methods 168, 1–8
31. Yamanaka, R., Lekstrom-Himes, J., Barlow, C., Wynah-boris, A., and Xanthopoulos, K. G. (1998) Int. J. Mol. Med. 1, 213–221
32. Kunisch, C., Lang, R. K., Rosen, C. A., and Shannon, M. F. (1994) J. Immunol. 153, 153–164
33. Trainor, C. D., Omichinski, J. G., Vanderberg, T. L., Gronenborn, A. M., Clure, G. M., and Felsenfeld, G. (1996) Mol. Cell. Biol. 16, 2238–2247
34. Pomerantz, O., Goodwin, A. J., Joye, T., and Lowrey, C. H. (1998) Nucleic Acids Res. 26, 5684–5691
35. Miyatake, S., Araí, N., and Araí, K. (2000) Int. Union Biochem. Mol. Biol. Life 49, 473–478

2 G. T. F. Schwenger, unpublished observations.
3 V. A. Mordvinov, unpublished observations.