Two Distinct Nuclear Factors Bind the Conserved Regulatory Sequences of a Rabbit Major Histocompatibility Complex Class II Gene

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The constitutive coexpression of the major histocompatibility complex (MHC) class II genes in B lymphocytes requires positive, *trans*-acting transcriptional factors. The need for these *trans*-acting factors has been suggested by the reversion of the MHC class II-negative phenotype of rare B-lymphocyte mutants through somatic cell fusion with B cells or T-cell lines. The mechanism by which the *trans*-acting factors exert their effect on gene transcription is unknown. The possibility that two highly conserved DNA sequences, located 90 to 100 base pairs (bp) (the A sequence) and 60 to 70 bp (the B sequence) upstream of the transcription start site of the class II genes, are recognized by the *trans*-acting factors was investigated in this study. By using the gel electrophoresis retardation assay, a minimum of two proteins which specifically bound the conserved A or B sequence of a rabbit DPβ gene were identified in murine nuclear extracts of a B-lymphoma cell line, A20-2J. Fractionation of nuclear extract through a heparin-agarose column allowed the identification of one protein, designated NF-MHCIIB, which bound an oligonucleotide containing the B sequence and protected the entire B sequence in the DNase I protection analysis. Another protein, designated NF-MHCIIA, which bound an oligonucleotide containing the A sequence and partially protected the 3' half of this sequence, was also identified. NF-MHCIIB did not protect a CCAAT sequence located 17 bp downstream of the B sequence. The possible relationship between these DNA-binding factors and the *trans*-acting factors identified in the cell fusion experiments is discussed.

Expression of the major histocompatibility complex (MHC) class II genes is essential for the generation of vertebrate immune responses. The MHC class II genes encode a family of related, membrane-bound glycoproteins composed of an alpha chain of 30,000 to 33,000 daltons and a beta chain of 27,000 to 29,000 daltons; these class II molecules are necessary for antigen recognition by helper T lymphocytes (17, 30). The expression of class II genes in normal individuals is strictly regulated in that constitutive expression is limited to a few cell types including mature B lymphocytes (11). In other cell types, expression requires an appropriate stimulus such as gamma interferon (6, 21, 32, 45), B-cell stimulatory factor 1 (BSF-1) (35), or gamma interferon plus lymphotoxin or tumor necrosis factor (36).

The molecular mechanism underlying the constitutive expression of the class II genes remains to be elucidated. In contrast to allelic exclusion of immunoglobulin genes, both alleles of all functional class II loci are simultaneously expressed in B lymphocytes (4, 17, 42, 44). This observation and the identification of a group of patients with severe, combined immunodeficiency (7, 25), as well as the identification of rare B-cell mutants (24, 26) which are unable to express multiple class II genes despite the presence of intact structural genes, suggest that there is a common pathway for transcriptional activation of all members of the gene family. Reversion of the class II-negative phenotype of human B-cell mutants, either by fusing with normal mouse spleen cells (1) or human T lymphocytes (38) or by transfecting with high-molecular-weight DNA (16), indicates that a part of the

common transcriptional pathway for class II genes is in the form of positive, *trans*-acting regulation.

All MHC class II genes contain two segments of conserved DNA sequences, A and B, located 90 to 100 base pairs (bp) and 60 to 70 bp, respectively, upstream of the transcription initiation site (22, 30, 43). Deletion of one or both of these conserved regions reduces transcriptional activity of the truncated gene upon transfection (3, 39). Other regulatory sequences observed in some class II genes, such as enhancer core sequence (14), octamer motif (39), and interferon type I responsive sequence (2, 12), are not conserved in all genes. Owing to the highly conserved nature of the A and B sequences, it has been suggested that they may be involved in the regulation of the coordinate expression of the class II genes (37). It is possible that the positive, *trans*-acting factors required for class II gene expression in B cells interact with the A and/or B segment. As a first step in testing this possibility, the gel electrophoresis retardation assay was used to identify and characterize soluble nuclear factors capable of binding to the conserved A and B sequences. The results indicate that there are at least two distinct nuclear factors in A20 B lymphoma cells that recognize these conserved sequences.

MATERIALS AND METHODS

DNA and labeling. A 200-bp *ApaI-PstI* DNA fragment encoding both conserved A and B sequences, a CCAAT box, and a short 5' portion of the proposed exon 1 of a rabbit DPβ gene was derived from a cosmid clone 18.2 (41; N. Sittisombut, J. Mordacq, and K. L. Knight, J. Immunol., in press). The nucleotide sequences of this 200-bp fragment and a 60-bp double-stranded synthetic oligonucleotide, which encodes the two conserved A and B sequences, are shown in

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FIG. 1. Nucleotide sequence of the 200-bp *ApaI-PstI* fragment of the rabbit DP β gene. The 60-bp synthetic oligonucleotide derived from the 200-bp *ApaI-PstI* fragment and three DNA fragments derived from restriction enzyme digestion of the 60-bp double-stranded oligonucleotide are represented as horizontal lines. The conserved A and B sequences and the CCAAT box are boxed. The restriction enzyme recognition sites are underlined. The proposed amino acid sequence of a 5' part of the first extracellular domain is shown in the three-letter code.

Fig. 1. A 105-bp *AvaI-PstI* fragment containing a VH octamer motif was derived from pRV10, a plasmid subclone of a rabbit VH gene segment (13). DNA fragments were end labeled with [γ - 32 P]ATP and T4 polynucleotide kinase (27).

Cells. A20-2J, a murine B lymphoma cell constitutively expressing the MHC class II genes, and HeLa cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum, penicillin, gentamicin, and amphotericin B at 37°C and 5% CO $_2$. WEHI-3, a murine myelomonocytic cell line which does not express class II genes, was maintained in Dulbecco modified Eagle medium supplemented with 5% fetal bovine serum, penicillin, and gentamicin.

Preparation of nuclear extracts. Crude nuclear extract was prepared from cells in log-phase growth essentially as described by Dignam et al. (8). For purification, crude extract (160 mg of protein) prepared from 19 ml of packed A20 cells was precipitated by addition of (NH $_4$) $_2$ SO $_4$ to 60% saturation at room temperature and centrifugation at 25,000 $\times$ g. The precipitate was suspended in 9 ml of TM buffer (50 mM Tris hydrochloride [pH 7.9], 12.5 mM MgCl $_2$, 1 mM EDTA, 1 mM dithiothreitol, 20% glycerol) (5) and applied to a Sephacryl S-300 column (25 cm by 80 cm; Pharmacia, Inc., Piscataway, N.J.) which was equilibrated with TM buffer containing 0.1 M KCl. The column eluate was monitored by A $_{280}$ and by the gel electrophoresis retardation assay. Active fractions were pooled and applied to a 10-ml DE-52 column (Whatman, Inc., Clifton, N.J.) equilibrated with TM buffer containing 0.1 M KCl. The DEAE-cellulose flowthrough fraction, which contained specific DNA-binding activity, was fractionated on a 5-ml Affi-Gel-heparin column (Bio-Rad Laboratories, Richmond, Calif.) equilibrated with TM buffer containing 0.1 M KCl. Bound materials were eluted with a step gradient of TM buffer containing 0.2 M, 0.3 M, and 0.4 M KCl and were diluted with TM buffer to a final salt concentration of 0.1 M. Fractionation on the Affi-Gel-heparin column resulted in two active fractions, the flowthrough fraction (30 mg of total protein) and the 0.3 M KCl eluate (12.5 mg of total protein), which bound the 200-bp *ApaI-PstI* fragment.

Gel electrophoresis retardation assay. Detection of specific binding activity was performed as described by Singh et al. (40). Typically, 5,000 or 10,000 cpm (1 ng) of [32 P]DNA was incubated with 800 to 2,000 ng of poly(dI-dC):(dI-dC) (Sigma Chemical Co., St. Louis, Mo., or Pharmacia) and nuclear

extract in 10 mM Tris hydrochloride (pH 7.5)–50 mM NaCl–1 mM dithiothreitol–1 mM EDTA–5% glycerol for 20 to 30 min at room temperature. The mixture was electrophoresed in a 3.5% polyacrylamide gel (acrylamide/bisacrylamide ratio, 30:1) or 7% gel (acrylamide/bisacrylamide ratio, 60:1) containing 6.7 mM Tris hydrochloride (pH 7.5), 3.3 mM sodium acetate, and 1 mM EDTA to separate bound and free DNA forms. The polyacrylamide gel was electrophoresed with buffer recirculating at 4 V cm $^{-1}$ following a preelectrophoresis for 30 min at 6 V cm $^{-1}$. After electrophoresis, the gel was dried and exposed at –70°C to X-ray films with an intensifying screen.

In competition experiments, unlabeled competitor DNA fragments were eluted from polyacrylamide gels and quantitated in ethidium bromide-stained gels by comparison with known amounts of *HindIII*-restricted λ DNA fragments.

DNase I protection analysis. Approximately 80,000 to 100,000 cpm of [32 P]DNA probe was incubated with 32 μ g of sheared salmon sperm DNA in 60 μ l of 50 mM Tris hydrochloride (pH 7.9)–12.5 mM MgCl $_2$ –1 mM dithiothreitol–1 mM EDTA–20% glycerol at 4°C for 10 min. Partially purified A20 nuclear extract (60 μ l) was added, and incubation continued at 4°C for 20 to 30 min. Limited digestion of DNA was initiated by addition of CaCl $_2$, MgCl $_2$, and freshly diluted DNase I (Worthington Diagnostics, Freehold, N.J.) to final concentrations of 5 mM, 10 mM, and 1.3 μ g/ml, respectively. After 1 min of digestion at room temperature, DNase I was inhibited by the addition of EDTA to a final concentration of 20 mM. The mixture was immediately electrophoresed in a 7% polyacrylamide gel (acrylamide/bisacrylamide ratio, 60:1) at 4°C as described for the gel electrophoresis retardation assay. Bound and free DNA bands were located by exposing the polyacrylamide gel to X-ray film at 4°C; DNA was eluted from the excised bands by shaking in 0.5 M ammonium acetate–1% sodium dodecyl sulfate–1 mM EDTA at 37°C overnight. The DNA was then purified by phenol-chloroform extraction, ethanol precipitation, and chromatographic separation on a NACS column (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). Equal amounts of radioactivity from DNase I-treated DNA eluted from the bound and free bands were analyzed by electrophoresis in 10 or 15% sequencing polyacrylamide gels and autoradiography.

RESULTS

Specific binding of the MHC class II conserved A and B sequences by A20 nuclear extract. Nuclear factors capable of recognizing the MHC class II conserved sequences were initially detected by mixing the 32 P-labeled 200-bp *ApaI-PstI* fragment of the rabbit DP β gene with a constant amount of nonspecific competitor, poly(dI-dC):(dI-dC) and increasing amounts of crude A20 nuclear extract. Upon electrophoresis and autoradiography, one prominent (B_1) retarded DNA band and one weak (B_2) retarded DNA band were observed (Fig. 2A). These retarded DNA bands were absent when the mixture was digested with proteinase K or heated at 65°C for 10 min, indicating that they represent a noncovalent complex of nuclear protein and labeled DNA (data not shown). Similar results were obtained with two other preparations of A20 nuclear extract.

To show that retardation of the 200-bp *ApaI-PstI* fragment was due to specific DNA-binding proteins, 10-fold and 100-fold molar excess of unlabeled DNA competitors were included in the mixture of 32 P-labeled 200-bp *ApaI-PstI* probe and poly(dI-dC) before the nuclear extract was added (Fig. 2B). The result showed that binding of the 200-bp *ApaI-PstI* fragment was effectively inhibited by unlabeled homologous competitor (Fig. 2B, lanes 2 and 3). A heterologous competitor containing the VH octamer motif of a rabbit VH gene segment did not affect the binding (Fig. 2B, lanes 4 and 5). In a reciprocal experiment, the VH octamer-containing fragment was labeled, and the interaction of this fragment with A20 nuclear protein was not inhibited by the 200-bp *ApaI-PstI* fragment but was inhibited by itself (Fig. 2C, lanes 1 to 5). Thus, under these conditions, the binding of A20 nuclear factors with the 200-bp *ApaI-PstI* fragment is specific.

The 200-bp *ApaI-PstI* fragment contained DNA sequences other than the conserved A and B segments (Fig. 1). To delineate the region within this 200-bp *ApaI-PstI* fragment which was recognized by A20 nuclear factors, a 60-bp oligonucleotide encoding the rabbit DP β conserved sequences was synthesized and used in the gel electrophoresis retardation assay. Interaction of A20 nuclear extract and this oligonucleotide resulted in a retarded band which migrated in the position corresponding to the B_1 band (Fig. 2D, lane 1, and Fig. 2E, lanes 1 and 4). In addition, if the binding reaction was prepared with a smaller amount of poly(dI-dC), a second retarded DNA band (B_2) was also observed (Fig. 2D, lane 2). The retarded B_2 band was not observed in experiments with the 60-bp oligonucleotide and may result from interaction of A20 factors with sequences outside the conserved regions.

Binding of the MHC class II conserved sequences by two distinct factors. To determine whether both the conserved A and B sequences can interact with the A20 nuclear factors, 32 P-labeled 60-bp oligonucleotide was restricted with *AluI* to obtain a 35-bp fragment and a 25-bp fragment, which contained the conserved A and B segments, respectively (Fig. 1). In the gel electrophoresis retardation assay, both DNA fragments were recognized by the A20 nuclear extract (Fig. 2E, lanes 6 and 8). In contrast, the A20 extract did not bind either a 280-bp unrelated DNA fragment or a 30-bp *BamHI-HindIII* polylinker fragment of M13mp18 DNA (Fig. 2E, lanes 2 and 10).

To assess the heterogeneity of the binding factors, A20 nuclear extract was first fractionated through the Sephacryl S-300 column. Analysis of S-300 fractions for specific binding activity with the 200-bp *ApaI-PstI* fragment showed that

FIG. 2. Detection of specific DNA-binding factors in A20 nuclear extract by gel electrophoresis retardation assay. (A) A mixture of 5,000 cpm of the 32 P-labeled 200-bp *ApaI-PstI* fragment and 1.6 μ g of poly(dI-dC) was incubated without nuclear extract (lane 1) or with nuclear extract at 1 μ g (lane 2), 2 μ g (lane 3), 3 μ g (lane 4), 6 μ g (lane 5), 12 μ g (lane 6), or 18 μ g (lane 7). (B) Binding competition experiment with the 200-bp *ApaI-PstI* fragment. A mixture of 4,000 cpm (0.5 ng) of the 32 P-labeled 200-bp *ApaI-PstI* fragment, 1.6 μ g of poly(dI-dC), and 6 μ g of nuclear extract was incubated with none (lane 1), 5 ng (lane 2), or 50 ng (lane 3) of the unlabeled 200-bp *ApaI-PstI* fragment or 2.7 ng (lane 4) or 27 ng (lane 5) of unlabeled 105-bp *Aval-PstI* fragment. (C) Binding competition experiment with the 105-bp *Aval-PstI* fragment. A mixture of 8,000 cpm (0.1 ng) of the 32 P-labeled 105-bp *Aval-PstI* fragment, 1.6 μ g of poly(dI-dC), and 6 μ g of nuclear extract was incubated with none (lane 1), 1 ng (lane 2), or 10 ng (lane 3) of the unlabeled 105-bp *Aval-PstI* fragment or 1.8 ng (lane 4) or 18.6 ng (lane 5) of the unlabeled 200-bp *ApaI-PstI* fragment. (D) 32 P-labeled 60-bp double-stranded oligonucleotide (5,000 cpm) was incubated with 6 μ g of nuclear extract and 2,000 ng (lane 1) or 400 ng (lane 2) of poly(dI-dC). (E) Mixtures of 800 ng of poly(dI-dC) and 5,000 cpm (0.2 to 22 ng) of the 32 P-labeled 200-bp *ApaI-PstI* fragment (lane 1), the 280-bp unrelated DNA fragment (lane 2), the 60-bp oligonucleotide (lanes 3 and 4), the 35-bp *AluI* fragment (lanes 5 and 6), and the 25-bp *AluI* fragment (lanes 7 and 8) of the 60-bp oligonucleotide and the 30-bp *BamHI-HindIII* fragment of mp18 DNA (lanes 9 and 10) were incubated with 6 μ g of nuclear extract (lanes 1, 2, 4, 6, 8, and 10) or without extract (lanes 3, 5, 7, and 9). Abbreviations: B, retarded DNA band; F, free DNA band.

the factor responsible for the B_2 band was eluted in the void volume and could be separated from the factor(s) responsible for the B_1 and B_3 bands which coeluted from the S-300 column (Fig. 3A). Because the B_2 band did not appear to be due to binding of A20 factors with the conserved regions, this factor was not further investigated. The S-300 fractions containing the factors responsible for the B_1 and B_3 bands were pooled and passed through DEAE-cellulose in 50 mM Tris hydrochloride (pH 7.9)–0.1 M KCl. Both binding activ-

FIG. 3. Separation of DNA-binding proteins by column chromatography. (A) A20 nuclear extract was fractionated on an S-300 column and monitored by A_{280} . DNA-binding activities were detected by the gel electrophoresis retardation assay with the 32 P-labeled 200-bp *Apal-PstI* fragment. (B) S-300 fractions 57 to 70 were pooled and applied to a DEAE-cellulose column. Specific DNA-binding activities in the DEAE-cellulose effluent were further fractionated on a heparin-agarose column. Separation of DNA-binding proteins was monitored by the gel retardation assay by incubating the labeled 200-bp *Apal-PstI* fragment and 800 ng of poly(dI-dC) without extract (lane 1) or with the DEAE-cellulose effluent (lane 2), the pooled heparin-agarose effluent (lane 3), the 0.2 M KCl heparin-agarose eluate (lane 4), the 0.3 M KCl heparin-agarose eluate (lane 5), or the 0.4 M KCl heparin-agarose eluate (lane 6). (C) Differential binding of the heparin-agarose fractions on the 35-bp *AluI* fragment and 25-bp *AluI* fragment of the 60-bp oligonucleotide. The 32 P-labeled 35-bp *AluI* fragment (lanes 1 to 4) and the 25-bp *AluI* fragment (lanes 5 to 8) were incubated without extract (lanes 1 and 5) and with the DEAE-cellulose effluent (lanes 2 and 6), the heparin-agarose effluent (lanes 3 and 7), or the 0.3 M KCl heparin-agarose eluate (lanes 4 and 8). Abbreviations: B, Retarded DNA band; F, free DNA band.

ities were eluted in the effluent and were applied to a heparin-agarose column in the same buffer. The nuclear factor which gave rise to band B_1 was found in the effluent, and the factor giving rise to band B_3 was eluted with 0.3 M KCl (Fig. 3B). To determine whether the two factors differed in sequence recognition, the effluent and 0.3 M KCl eluate were tested on *AluI*-restricted fragments of the 60-bp oligonucleotide. The effluent fraction bound the 25-bp fragment, but not the 35-bp fragment; the 0.3 M KCl eluate bound the 35-bp fragment, but not the 25-bp fragment (Fig. 3C). Therefore, a minimum of two nuclear proteins distinct in chromatographic properties and binding specificities have been identified. One of these proteins, designated NF-MHCIIA, bound a DNA fragment containing the MHC class II conserved A segment, and the other protein, designated NF-MHCIIB, bound the B segment.

DNase I protection analysis by NF-MHCIIA AND NF-MHCIIB. The regions of DNA on the 200-bp *Apal-PstI* fragment on the 60-bp oligonucleotide which were bound by NF-MHCIIA and NF-MHCIIB were located more precisely by DNase I protection analysis. On the 200-bp *Apal-PstI* fragment, NF-MHCIIB protected two sites on the coding strand, 4 and 6 bp long, separated by 7 bp of unprotected bases (Fig. 4A). Protection at the proximal 4-bp site was more pronounced on the 60-bp oligonucleotide (Fig. 4B). In addition, five more bases were protected at the distal site on the 60-bp oligonucleotide by NF-MHCIIB (Fig. 4B); the reason why these five bases were not protected on the 200-bp *Apal-PstI* fragment is not known. On the noncoding strand of the 200-bp *Apal-PstI* fragment, NF-MHCIIB protected a 14-bp stretch of DNA which overlapped the distal site on the coding strand (Fig. 4D). The two sites bound by

NF-MHCIIB included a 3' part of intervening region between the two conserved regions and the conserved B sequence (Fig. 5A). NF-MHCIIB did not protect a CCAAT sequence located 17 bp downstream, although this CCAAT site shared the canonical CCAAT sequence with the conserved B sequence (Fig. 4A).

NF-MHCIIA partially protected a 20-bp region on the coding strand and a 17-bp region on the noncoding strand (Fig. 4C and E). The boundary of protection at the 3' end of the noncoding strand was delineated by a hyperdigested base seen in each of two experiments. This partially protected 17- to 20-bp region corresponded to the 3' half of the conserved A sequence and the 5' part of the intervening region (Fig. 5A).

Binding of the conserved A and B sequences by HeLa and WEHI-3 nuclear factors. To determine whether nuclear proteins capable of binding the class II conserved A and B sequences were present in cells that do not express the class II genes, nuclear extracts prepared from HeLa cells and WEHI-3 cells were tested on the gel electrophoresis retardation assay with the 32 P-labeled 35-bp *AluI* fragment and the 35-bp *HinfI* fragment containing the conserved A and B sequences, respectively. Binding activities for both DNA fragments were detected in the HeLa extract and in the WEHI-3 extract (Fig. 6). The number and positions of retarded DNA bands resulting from the WEHI-3 extract were quite different from those of the A20 extract and HeLa extract.

DISCUSSION

Although the highly conserved nature of the A and B sequences of the MHC class II genes was noticed in 1983 (37), it is only recently that Boss and Strominger (3) and Sherman et al. (39) have shown that the A and B regions are required for efficient gene transcription. The molecular mechanism by which the conserved sequences are involved in the transcriptional regulation was first explored by Sherman et al. (39). By using the gel electrophoresis retardation competition assay, Sherman et al. (39) clearly demonstrated the specific binding of the conserved B region of the *HLA-DR α* gene by nuclear proteins. In the DNase I protection analysis, Miwa et al. (31) found an additional binding at the conserved A sequence of the *HLA-DQ2 β* gene. The binding at the A region is comparatively weaker than at the B region. In these experiments, it was not possible to tell whether the conserved A and B binding activities reside in the same or different proteins. Moreover, the extent of binding has not been established. Most recently, Dorn et al. (9, 10) extensively characterized a nuclear factor in the extract of the B lymphoma M12 line that binds the conserved B region of the *H-2E α* gene. Because they could not clearly demonstrate the binding at the conserved A region with these extracts, it can be inferred only that distinct proteins recognized the conserved A and B regions of the *E α* gene. In this study, I was able to separate, by heparin-agarose column chromatography, two DNA-binding nuclear proteins designated NF-MHCIIA and NF-MHCIIB, which are present in the A20 murine B-lymphoma crude nuclear extract. In DNase I protection analysis, the partially purified preparations of NF-MHCIIA and NF-MHCIIB protected the 3' half of the conserved A sequence and the entire B sequence, respectively, of the rabbit *DP β* gene. Separation of these two factors establishes that binding at the conserved A and B regions of the rabbit *DP β* gene is due to a minimum of two chromatographically distinct proteins and that the conserved

FIG. 4. DNase I protection analysis by NF-MHCIIA and NF-MHCIIB. DNA fragments (the 200-bp *ApaI-PstI* fragment [panels A, C, and D] or the 60-bp oligonucleotide [panels B and E]) were 32 P labeled on the coding strand (panels A, B, and C) or the noncoding strand (panels D and E) and were used in the analysis with NF-MHCIIB (panels A, B, and D) and with NF-MHCIIA (panels C and E). DNase I-digested products eluted from the bound (lanes BOUND) or free (lanes FREE) DNA bands were analyzed on a 10 or 15% sequencing gel with the same fragments cleaved at purine residues (lanes G+A). In a high percentage gel, the G+A reaction products migrated slightly slower than DNase I-digested products owing to an extra phosphate group. The conserved A and B sequences, a CCAAT box, and the protected regions are indicated.

A and B sequences can independently interact with sequence-specific nuclear proteins.

Somatic cell fusion experiments with class II-negative human B-lymphocyte mutants and normal murine spleen cells or human T-cell lines suggest that coordinate expression of the MHC class II genes in B cells requires positive, *trans*-acting transcriptional factors (1, 38) which can act across species barriers. Because the *trans*-acting factors simultaneously activate transcription of several class II genes, it is likely that they interact with the *cis*-acting element(s) common to all members of this gene family. Up to now, the A and B sequences have been the only known positive-control, *cis*-acting elements whose sequences and positions are well conserved in all MHC class II α and β genes. These A and B sequences are also present in the invariant chain gene which is constitutively coexpressed and is coordinately induced with the class II genes (23, 33, 34). It is very likely that the *trans*-acting factors interact, either directly or indirectly, with the conserved A and B sequences. If this is the case, NF-MHCIIA and NF-MHCIIB can represent the *trans*-acting factors or are required for the indirect interaction of the *trans*-acting factors with the A and B sequences.

Miwa et al. (31) have provided the evidence from the gel electrophoresis retardation experiments that nuclear extract from the two class II-negative B-cell mutants and their respective parental lines do not differ in the DNA-binding activities when tested on up to 4 kilobases of DNA from the *HLA-DQ2 β* promoter which includes the conserved A and B regions. If NF-MHCIIA and NF-MHCIIB represent the *trans*-acting factors, their results suggest that the mutation in

the class II-negative cells does not affect the DNA-binding function of the *trans*-acting factors. Alternatively, this finding is consistent with the possibility that the *trans*-acting factors interact indirectly with the conserved A and B sequences.

Although nuclear factors capable of binding to the conserved A and B sequences can be detected in the class II-nonexpressing cells, the retarded DNA bands resulting from WEHI-3 nuclear factor binding appear to be different from those resulting from A20 nuclear factor binding. This difference is not due to polymorphism, because WEHI-3 and A20 are both derived from BALB/c strain. On the other hand, binding of the A- and B-containing oligonucleotides by HeLa cell extract results in retarded bands quite similar to those resulting from A20 extract. It is unlikely that the WEHI-3 binding patterns were due to nonspecific binding of murine protein with two oligonucleotides, since the retardation patterns are quite different, although the sizes of the oligonucleotides and the binding conditions were identical. Although it is clear that cells differ with respect to the class II-specific DNA-binding proteins, how or whether these proteins are involved in the regulatory process remains to be elucidated. Structural and functional characterization of the purified proteins is required to help solve this problem.

The relationship of NF-MHCIIA and NF-MHCIIIB to other DNA-binding transcriptional factors is not clear. Because the MHC class II A and B regions do not exert strong enhancing activity at a distance, but are required for efficient transcription, they resemble the upstream promoter elements more than they resemble the enhancer elements (28). Although there is no strong sequence similarity of the consensus A sequence to any well-characterized upstream promoter element (such as GGGCGG, CCAAT, ATGCAAT, or GCCACACCC), the consensus B sequence contains the canonical CCAAT pentanucleotide in the reverse orientation.

Many eucaryotic promoters contain one or more inverted CCAAT sequences, and for the herpes simplex virus thymidine kinase gene, this sequence can be recognized by two

transcriptional factors, CCAAT transcription factor (CTF) and CCAAT binding protein (CBP), distinct in chromatographic properties and DNase I protection patterns (15, 19, 20). These factors appear to be members of a family of proteins which recognize the CCAAT sequence. The factor NF-MHCIIB, which recognizes the MHC class II B region, generates a DNase I protection pattern quite different from that of CTF and CBP. It is possible that this factor represents the mouse homolog of CTF and CBP and that the difference in protection pattern reflects species or template differences. However, it is more likely that NF-MHCIIB represents another member of the CBP family. In support of this possibility are (i) our observation that NF-MHCIIB selectively protected the CCAAT sequence of the class II B region, but not the similar CCAAT sequence located just 17 bp downstream and (ii) the finding of Miwa et al. (31) that even in the homologous system, protection at the conserved

FIG. 6. Detection of DNA-binding activities in HeLa cells and WEHI-3 cell extracts. The ^{32}P -labeled 35-bp *AluI* fragment (lanes 1 to 4) and the 35-bp *HinfI* fragment (lanes 5 to 8) of the 60-bp oligonucleotide were incubated without extract (lanes 1 and 5) and with extracts from A20 cells (lanes 2 and 6), HeLa cells (lanes 3 and 7) or WEHI-3 cells (lanes 4 and 8) and analyzed by gel electrophoresis. Abbreviations: B, bound DNA band; F, free DNA band.

B region can occur without simultaneous protection of the nominal CCAAT sequence. It has been observed that the nucleotide sequence of several bases surrounding the CCAAT pentanucleotide of the MHC class II B region is well conserved, but is quite different from its counterparts in other CCAAT regions, including that of the herpes simplex virus thymidine kinase gene (Fig. 5B). The nucleotides surrounding the CCAAT sequence may play an important role in DNA-protein interaction, and it may be that the nucleotides surrounding the CCAAT sequence determine which member of the CBP family will bind a particular CCAAT site. Recently, Dorn et al. (9) showed conclusively that NF-Y, which recognized the conserved B region of the E α gene, is distinct from CTF and CBP. The relationship between NF-Y and NF-MHCIIB remains to be elucidated.

Further studies of NF-MHCIIA and NF-MHCIIB with respect to structural and functional properties should help us to better understand the mechanism by which the MHC class II gene expression is regulated. Because the quantitative variation of cell surface class II molecules influences the magnitude of the immune response (18, 29), the study of control mechanism(s) may provide an effective means of manipulating the immune response of healthy and diseased individuals.

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