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Cell-Specific Expression of the Murine CD21 Gene Depends on Accessibility of Promoter and Intronic Elements

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The murine complement receptor type 2 gene (Cr2, CD21) is transcriptionally active in murine B and follicular dendritic cells, but not in murine T cells. We have previously shown that altering chromatin structure via histone deacetylase inhibitors results in CD21 expression in murine T cells, and that the minimal CD21 promoter provided appropriate cell-specific expression of luciferase reporter constructs only in the presence of the first third of intron 1, fragment A. We extend this work by showing that replacing the CD21 gene promoter with the SV40 promoter resulted in the loss of this cell-specific control. Further delineation of intronic regulatory elements by fragmentation also resulted in the loss of cell-specific gene expression, suggesting that multiple CD21 promoter and intronic elements interact for appropriate CD21 gene expression. To assess this model, we performed EMSAs to define protein binding sites within promoter and intronic regions and DNase I hypersensitivity assays to determine chromatin accessibility. Multiple DNA binding factors were shown to be present in B and T cell extracts; a minority demonstrated B cell specificity. However, the DNase I sensitivity of T cell CD21 regulatory elements was not comparable to that of B cells until the histone acetylation status of the gene was altered. Taken together, these data suggest that chromatin remodeling facilitates cell-specific CD21 gene expression by modulating access of transcription factors to regulatory elements in the promoter and intron.

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We have demonstrated that murine T cells can be induced to express CD21 by altering the conformation of the chromatin (17). Treatment with the histone deacetylase inhibitors sodium butyrate and trichostatin A (TSA)3 induced murine T cells to express CD21, suggesting that histone positioning contributes to the control of CD21 expression, and that certain regulatory elements that have been mapped within the gene may function via this pathway.

We have previously identified the minimal CD21 promoter and intronic regulatory elements within fragment A that control the cell-specific expression of the murine CD21 gene. In this manuscript we describe the dependence of these intronic elements on the endogenous CD21 promoter for cell-specific gene expression. In addition, we show that segmentation of fragment A results in the loss of cell-specific CD21 gene expression. Taken together, these data indicate that multiple regulatory elements in fragment A interact with the endogenous promoter to confer cell-specific expression on the murine CD21 gene.

Two different models can be proposed to explain these data. The first involves regulating CD21 gene expression by controlling the expression of transcription factors that bind CD21 promoter and intronic regulatory elements. A second model involves regulating CD21 gene expression by controlling the interaction with the endogenous promoter to confer cell-specific expression. Nuclear extracts were prepared from 2PK3 and EL4 cells, and all steps were performed at 4°C. Briefly, 5 × 106 cells were resuspended in 0.65 ml of DMEM with 10% FCS and 1% Pen-Strep and incubated at 37°C for 48 h. Luciferase assays were performed using the Dual Luciferase Reporter Assay System (Promega). Briefly, cells were harvested, centrifuged at 200 × g for 5 min, washed twice in PBS, resuspended in 200 μl of 1 x lysis buffer (Promega), and incubated at room temperature for 15 min. Cell debris was pelleted by centrifugation at 13,000 × g for 5 min at 4°C. Lysate was removed. Cell lysate (10 μl) was loaded into the well of a white opaque microtiter plate, and the dual luciferase assay was performed automatically using the MLX microtiter plate luminometer (Dynex, Chantilly, VA). Sialic acid was removed by centrifugation at 13,000 × g for 10 min, and nuclear extract was removed and quantified by the Bradford assay (20). DNA fragments were prepared by PCR using primers specific for each segment of fragment A and the promoter. Primers used for amplifying fragment A segments i1–9 are the same as those used in cloning the luciferase reporter constructs. In addition, i4 was amplified using primers 1250 (5'-CTG TTT CCT CTG TCT C-3') and 1251 (5'-CCT GAT CCC TCT ACT CCT C-3') and 1162 (5'-GTG TTA GAT TAT TAT TAT GTA C-3'), p4 was amplified using primers 1163 (5'-TGT CCT AAA TTT ATT TAA AAA TCT GGA A-3') and 1164 (5'-TAA AGT GGA AAT TCA ACA ACA ATC C-3').

Luciferase reporter constructs

Mouse 2PK3 cells, CD21-expressing B lymphomas, and EL4 cells, CD21-nonexpressing T lymphomas, were obtained from American Type Culture Collection (Manassas, VA). 2PK3 cells were maintained at 37°C in 5% CO2 in DMEM (Life Technologies, Gaithersburg, MD) with 10% FCS (HyClone, Logan, UT) and 1% penicillin-streptomycin (Pen-Strep, Life Technologies). EL4 cells were maintained at 37°C in 5% CO2 in RPMI 1640 (Life Technologies) with 10% FCS and 1% Pen-Strep. Where indicated, cells were incubated with 100 nM of the deacetylase inhibitor TSA (Sigma, St. Louis, MO) overnight.

Materials and Methods

Cell lines and culture conditions

Cell transfection and assay of luciferase reporter constructs

Transfections were performed as previously described (7). Briefly, 5 × 105 2PK3 or EL4 cells were resuspended in 0.65 ml of DMEM with 10% FCS and 1% Pen-Strep. Equimolar amounts of plasmid were added relative to 10 μg of pGL3Basic luciferase reporter construct (Promega). One microgram of pRL-TK Renilla luciferase plasmid (Promega) was added to control for transfection efficiency. Cells were incubated with plasmids for 5 min in 0.4-cm electrode gap cuvettes (Invitrogen, Carlsbad, CA) and electroporated at room temperature using the Gene Pulser (Bio-Rad, Hercules, CA) at 250 V and 960 μF for 2PK3 cells and at 280 V and 960 μF for EL4 cells. Cells were transferred to 100 × 20-mm tissue culture dishes containing 10 ml of DMEM with 10% FCS and 1% Pen-Strep and incubated at 37°C for 48 h. Luciferase assays were performed using the Dual Luciferase Reporter Assay System (Promega). Briefly, cells were harvested, centrifuged at 200 × g for 5 min, washed twice in PBS, resuspended in 200 μl of 1 x lysis buffer (Promega), and incubated at room temperature for 15 min. Cell debris was pelleted by centrifugation at 13,000 × g for 5 min at 4°C. Lysate was removed. Cell lysate (10 μl) was loaded into the well of a white opaque microtiter plate, and the dual luciferase assay was performed automatically using the MLX microtiter plate luminometer (Dynex, Chantilly, VA). Sialic acid was removed by centrifugation at 13,000 × g for 10 min, and nuclear extract was removed and quantified by the Bradford assay (20). DNA fragments were prepared by PCR using primers specific for each segment of fragment A and the promoter. Primers used for amplifying fragment A segments i1–9 are the same as those used in cloning the luciferase reporter constructs. In addition, i4 was amplified using primers 1250 (5'-CTG TTT CCT CTG TCT C-3') and 1251 (5'-CCT GAT CCC TCT ACT CCT C-3') and 1162 (5'-GTG TTA GAT TAT TAT TAT GTA C-3'), p4 was amplified using primers 1163 (5'-TGT CCT AAA TTT ATT TAA AAA TCT GGA A-3') and 1164 (5'-TAA AGT GGA AAT TCA ACA ACA ATC C-3').

Electrophoretic mobility shift assays

The protocol was performed as previously described (19) with minor modifications. Nuclear extracts were prepared from 2PK3 and EL4 cells, and all steps were performed at 4°C. Briefly, 5 × 106 cells were incubated with 100 nM of the deacetylase inhibitor TSA (Sigma, St. Louis, MO) overnight.

3 Abbreviations used in this paper: TSA, trichostatin A; HS, hypersensitivity; gDNA, genomic DNA.
5 mM MgCl₂, 0.5 mM DTT, 0.5 mM EDTA, and 3% glycerol). Reactions were subjected to 4% PAGE for 2 h at 250 V. Gels were dried and exposed to XAR medical x-ray film (Western X-ray, Salt Lake City, UT) for 3–16 h.

**DNase I hypersensitivity PCR (DNase I HS PCR)**

This assay is based on modifications of previous protocols (21, 22). Briefly, 10⁶ 2PK3 and EL4 cells were harvested, centrifuged, and washed twice with PBS. Cells were resuspended in 1 ml of permeabilizing buffer (15 mM Tris (pH 7.5), 60 mM KCl, 15 mM NaCl, 5 mM MgCl₂, 0.5 mM EGTA, 300 mM sucrose, and 0.5 mM 2-ME) supplemented with 0, 500, 2,000, or 10,000 U/ml of DNase I (Roche, Indianapolis, IN). An equal volume of permeabilizing buffer supplemented with 0.1% lysolceitin (Sigma) was added, and the reaction was incubated at room temperature for 4 min. Genomic DNA (gDNA) was isolated using the DNEasy Tissue Kit (Qiagen, Valencia, CA). PCR was performed in 10-µl reactions using 10 ng of genomic DNA (gDNA), 70 µl of each primer, 0.72 U of AmpliTaq DNA polymerase (Life Technologies), 0.8 mM dNTPs, 1× Taq buffer (50 mM Tris (pH 8.3), 3 mM MgCl₂, 20 mM KCl, and 500 µg/ml BSA), and 2.5 mM [α-³²P]dCTP. Samples were loaded into capillary tubes and incubated in an air thermocycler (Idaho Technologies, Idaho Falls, ID) for denaturing at 94°C for 1 s, for annealing at 60°C for 1 s, and for extension at 72°C for 5 s. This cycle was repeated 25 times for amplifying β-actin sequences from 2PK3 and EL4 gDNA with primers 62 (5’-ATT GAA CAT GGC 9) and 339 (5’-CTC TAT CGT GGG CCG CTC TAG-3’). The cycle was repeated 28 times for amplifying promoter and fragment A segments from 2PK3 and EL4 gDNA with the same primers as those used to generate EMSA probes. For gDNA isolated from TSA-treated EL4 cells, the cycle was repeated 28 times for amplifying β-actin sequence and 31 times for amplifying promoter and fragment A segments. After amplification, 5 µl of stop solution (U.S. Biochemical, Cleveland, OH) was added to the reactions, and 5 µl was resolved on a 6% acrylamide sequencing gel, which was then dried and exposed to x-ray film (Western X-ray) overnight. PCR bands visualized on x-ray film were subsequently visualized using a Bio-Rad GS-250 phosphorimager and were quantified using Bio-Rad Molecular Analyst software version 1.1. Quantified band intensity values were normalized by β-actin values and relativized to band intensity values for PCR using genomic DNA without DNase I treatment. Statistical analysis was performed using Microsoft Excel.

**Results**

**Cell specificity of CD21 intronic regulatory elements is promoter dependent**

We have previously shown that the cell-specific expression of murine CD21 (B cells->T cells) is not regulated by 5’ promoter sequences (7), and sequences have been described within the first third of intron 1, termed fragment A (Fig. 1), that do confer this cell-specific expression. Fragment A, with the CD21 promoter, increased the expression of a luciferase reporter construct in B cells, but not in T cells. The 5’ half of fragment A, termed Aa, repressed luciferase reporter activity in both B and T cells, while the 3’ half of fragment A, termed Ab, enhanced luciferase reporter activity. Taken together, these data suggest that fragment A contains multiple sites that can positively and negatively affect CD21 transcription, neither of which recapitulates full fragment A function. Of particular interest in this region was segment 8 of fragment Ab, which contains the MHI sequence that possesses high homology to the human CD21 intron 1 (Fig. 1). Sequence conservation between the mouse and human in a noncoding region of CD21 implies an important regulatory role for the i8 segment.

To further elucidate the function of these intronic elements, we examined whether fragment A required the endogenous CD21 promoter to confer cell-specific expression, or whether this intronic element would confer similar control with an exogenous promoter. We cloned CD21 intronic elements into a luciferase reporter construct driven by an SV40 promoter with an SV40 enhancer sequence (3’. The positions of the mouse/human homology sequence (MHP), the octamer binding site (Oct), and the TATA box relative to the segments are indicated. The first third of intron 1 (segment A) is shown divided into two pieces of ~800 bp (Aa and Ab). Both of these are further subdivided into segments of ~200 bp each (i1–i9). The relative position of the mouse/human homology sequence is shown within i8.

**Cell-specific CD21 gene expression is lost when fragment A is segmented**

The previous experiments revealed that the cell-specific expression conferred by fragment A appears to be adversely affected when it is fragmented. Thus, while Aa and Ab appear to have negative and positive regulatory elements, respectively, neither fragment confers the cell specificity that fragment A does. It was possible that hidden effects might be displayed by further reducing the size of the fragment tested. Therefore, we expanded this analysis with reporter constructs using nine different overlapping segments (i1–i9) of ~200 bp each (Fig. 1) derived from fragment A. Each of...
these segments was amplified from intron 1 by PCR and cloned into pCD21Basic. These constructs were then transiently transfected into 2PK3 and EL4 cells, and luciferase assays were performed (Fig. 3). Segments i1–i5 (together constituting the Aa fragment) did not demonstrate the repressor activity that was clearly evident with the full-length Aa fragment. Segment i4 proved refractory to several cloning attempts due to a repetitive motif. However, pCD21Basic i3–i5, which contained the i4 sequence, was cloned and transfected into 2PK3 and EL4 cells. Luciferase assay results were similar to those seen for the other Aa segments (data not shown). Similarly, segments i6–i9 (together constituting the Ab fragment) did not exhibit the enhancer activity that was clearly evident from full-length Ab. In fact, i7 and, to a lesser extent, i8, strongly repressed luciferase expression. These data suggest that fragment A represents a cell-specific regulatory unit that contains many sequences that coordinately are required for its proper function. If one or more of these sequences is altered, then so is the cell-specific expression of the CD21 gene.

CD21 fragment A contains many nuclear protein binding sites

The previous experiments demonstrated that functional CD21 regulatory elements exist in fragment A. To determine the existence and approximate locations of NF binding sites that may constitute these regulatory elements, we performed EMSA analysis with fragment A sequences (Fig. 4). The same segments of fragment A used in the functional assays (i1–i9) were incubated with nuclear extracts from 2PK3 (B cell) and EL4 (T cell) and luciferase assays were performed (Fig. 3). Segments i1–i5 (together constituting the Aa fragment) did not demonstrate the repressor activity that was clearly evident with the full-length Aa fragment. Segment i4 proved refractory to several cloning attempts due to a repetitive motif. However, pCD21Basic i3–i5, which contained the i4 sequence, was cloned and transfected into 2PK3 and EL4 cells. Luciferase assay results were similar to those seen for the other Aa segments (data not shown). Similarly, segments i6–i9 (together constituting the Ab fragment) did not exhibit the enhancer activity that was clearly evident from full-length Ab. In fact, i7 and, to a lesser extent, i8, strongly repressed luciferase expression. These data suggest that fragment A represents a cell-specific regulatory unit that contains many sequences that coordinately are required for its proper function. If one or more of these sequences is altered, then so is the cell-specific expression of the CD21 gene.
particularly interesting because it includes the MHI region (Fig. 1). Both nuclear extracts failed to form complexes with segments i6 and i9 (data not shown). We did not observe any T cell-specific complexes with any of the segments. These data in combination with the reporter assays suggest that fragment A of intron 1 regulates the cell-specific expression of CD21 by binding factors present in both T and B cells as well as those uniquely present in B cells.

**Differential CD21 promoter EMSA patterns with B and T cell nuclear extracts**

The data presented in Fig. 2 indicated that the CD21 promoter is required for cell-specific regulation by fragment A sequences, suggesting that B cell-specific NF binding sites may be present within this promoter. To determine whether the CD21 promoter does possess differences in B and T cell nuclear extract binding, we performed EMSA analysis on the proximal 1200-bp promoter sequence. We generated six overlapping segments (p6–p1) from the CD21 promoter, each ~200 bp (Fig. 1). Potentially important sites for transcriptional control include an octamer binding site as well as a sequence with high homology to the human CD21 promoter, termed MHP, in p2. In addition to the TATA box, an Sp1 site is located in p1. Identical banding patterns were observed for both 2PK3 and EL4 nuclear extracts with segments p5 and p4, although the 2PK3 nuclear extracts appeared to produce a much stronger p4b complex than the EL4 nuclear extract (Fig. 5). Band shift complexes p3b, p2b, p1a, p1c, and p1d were also identical with both extracts. However, three other complexes appeared to be unique to the B cell nuclear extract: p3a, p2a, and p1b. Complex p2a confirms previous data in which the B cell nuclear extract, which contains both Oct-1 (ubiquitous) and Oct-2 (B cell-specific) factors, forms a complex with fragment A.

**FIGURE 5.** EMSA analysis of the CD21 promoter. Binding reactions were performed as previously described. The lettered arrows indicate shifts for each fragment. Fragment p6 produced no shift (data not shown).
independent experiments. Fragment listed for any given set. Data are the averages of at least three experiments. SD error bars are shown for only the first fragment listed for any given set. Data are the averages of at least three experiments.

transcription factors, produces two band shifts when it binds the octamer binding site located in p2 of the CD21 promoter (23). These data suggest that the CD21 promoter possesses regulatory elements capable of binding lymphoid and B cell-specific transcription factors that may cooperate with fragment A regulatory elements to control cell-specific CD21 gene expression.

**Differential DNase I susceptibility of CD21 regulatory elements in T and B cells: alteration by histone acetylation**

We have previously shown that murine T cells can be induced to express CD21 by increasing the acetylation state of core histones (17). CD21 transcript and protein were detected in and on T cells treated with TSA, suggesting that the MHCIIA$\alpha$ gene is hypersensitive to DNase I treatment in T cells (26). In our assay the MHCIIA$\alpha$ enhancer was sensitive in B cells after treatment with 2000 U/ml of DNase I, but was still detected in T cells treated with as much as 10,000 U/ml of DNase I. Conversely, the TCR$\beta$ enhancer was not sensitive in B cells treated with as much as 10,000 U/ml of DNase I, but was sensitive in T cells after treatment with as little as 2,000 U/ml of DNase I. Any DNA segment that was sensitive with the 2,000 U/ml DNase I treatment was termed ultrasensitive, and those sensitive with 10,000 U/ml were termed hypersensitive. Thus in Fig. 6, the p1 segment of the CD21 promoter is ultrasensitive to DNase I within the B cell nucleus, resistant in the T cell nucleus, and hypersensitive in the nuclei of T cells treated with TSA. Interestingly, the MHCIIA$\alpha$ enhancer became hypersensitive to DNase I treatment in T cells treated with TSA, suggesting that the MHCIIA$\alpha$ gene may also be positively regulated by histone hyperacetylation. Taken together, these data indicate that the assay is sensitive enough to assess DNase I accessibility to DNA regulatory elements.

We further assessed the DNase I hypersensitivity of all CD21 promoter and fragment A segments, quantifying PCR band intensities by phosphorimage analysis to more accurately interpret the results. Most of the CD21 promoter was hypersensitive in B cells, where CD21 is transcriptionally active (Fig. 7). In B cells p6–p3 were hypersensitive, while p1, the sequence closest to the transcriptional start site, was ultrasensitive in B cells. Conversely, none of the CD21 promoters was hypersensitive in T cells. However, upon treatment of T cells with TSA, p6, p5, p3, and p1 all became hypersensitive. This trend continued when we analyzed CD21 fragment A segments; i2–i4, i7, and i9 were hypersensitive, while i8 was ultrasensitive. Interestingly, i9 was the only hypersensitive site identified in T cells. When T cells were treated with TSA, i2, i4, and i6–i8 became hypersensitive, while i8 was no longer hypersensitive (Fig. 8). Thus, the hypersensitivity profile of the CD21 gene in T cells changes dramatically when chromatin is remodeled, closely matching the B cell profile. These data suggest that chromatin structure tightly regulates accessibility to CD21 regulatory elements in a cell-specific manner, allowing for, in a permissive state, transcriptional induction.

**Discussion**

We and others have previously shown that intronic elements control the cell-specific expression of both murine and human CD21 genes (7, 8). These data are similar to those for human and mouse.
CD4, which also demonstrated intronic control of cell-specific expression (10, 11). In this study we present functional data that expand our understanding of intronic and promoter regulatory components, and structural data that define factor binding sites and the accessibility of CD21 sequences in the nuclei of expressing and nonexpressing lymphocytes.

Previous analyses of CD21 intronic control were conducted only with the CD21 promoter (7). Enhancer function is simplistically defined as promoter independent, in that a defined enhancer element can increase the transcriptional activity of a variety of different promoters. Thus, the question was raised: can the CD21 intronic elements regulate the activities of other promoters or is the cell-specific expression control specific for the CD21 promoter? As shown in Fig. 2, fragment A, the first 1.6 kb of intron 1 that bestowed cell-specific expression of CD21 reporter constructs failed to direct appropriate expression of the reporter gene when controlled by an SV40 promoter. Only when the CD21 promoter was used in these constructs was the cell-specific expression restored, suggesting that specific protein/sequence associations must occur between the promoter and intronic elements for appropriate cell-specific control. Of future interest will be the creation of multiple enhancer and suppressor elements that cooperate for appropriate transcriptional control. Intronic sequences containing both enhancer and silencer elements have been described for many genes, including the murine keratin 18 gene (27) and the β3 tubulin gene in Drosophila (28). In both these cases, appropriate gene expression required the combined activity of all positive and negative intronic regulatory elements as well as the endogenous promoter. The murine CD21 gene appears to require similar cooperation among its many regulatory sequences.

To further elucidate the mechanism by which intronic and promoter elements control the cell-specific expression of the murine CD21 gene, we mapped nuclear protein binding sites. EMSA analysis with T and B cell nuclear extracts revealed many factor binding sites. Additionally, there are multiple sites unique for B cell factor binding, but none specific for T cell binding (Fig. 9). While any or all of these binding sites may be important for modulating cell-specific gene regulation, those that demonstrate B cell-specific binding are of particular interest. In the intron, two of these specific band shifts (i8a and i8b) reside in an area containing ~80 bp of sequence homologous to human intron 1 that repressed reporter gene expression (Fig. 4). This region is found in the same relative location in both mouse and human first introns and has been shown to be part of a silencer in the human CD21 gene (8). Paradoxically, the entire Ab fragment, of which i8 is only a part, does not demonstrate suppression in similar reporter assays (Fig. 2). These data suggest that additional enhancer or suppressor inhibitory elements exist within this sequence.

In the CD21 promoter region, three B cell-specific nuclear protein band shifts were identified, including one in the region containing the transcriptional start site (p1b, Fig. 5). A 200-bp region 5′ to the TATA containing region also produced a B cell-specific complex (p3b). Between these two regions another B cell-specific band shift (p2b) was produced in a region in which we had previously identified an octamer binding site (23). This site, however, was not capable of binding the B cell-specific OcaB transcription factor and, therefore, would not alone confer B cell-specific CD21 gene expression (29–32). A small region of homology to the human CD21 promoter, MHP, is located in p2, but no additional bands were seen that would suggest binding of additional NFs to this site.

Data collected from EMSAs reveal significant differences in the binding of components of B and T cell nuclear extracts to the CD21 promoter and intronic elements that may explain the cell-specific regulation of the gene. However, EMSAs only reveal the capability of nuclear proteins to bind naked DNA fragments and retard their movement, which may not be physiologically relevant. Performing an in vivo DNase I-hypersensitive PCR allowed us to investigate the accessibility of NFs to putative CD21 promoter and intronic regulatory elements identified by EMSAs. This assay proved to be sensitive enough to classify hypersensitivity to different degrees, which were arbitrarily defined based on the amount of DNase I treatment that prevented PCR amplification.

A strikingly different hypersensitivity profile was found in the CD21-expressing B cell compared with that of the nonexpressing T cell. Most of the promoter and fragment A was hypersensitive in B, but not T, cells. When we induced a hyperacetylated state in T cells, however, the hypersensitivity profile changed to one nearly identical with that seen in the B cell. DNase I hypersensitivity PCR data can be compared with the EMSA data to discriminate between potentially relevant and irrelevant bindings (Fig. 9). Those segments that displayed both binding by B cell-specific NFs and B cell DNase I hypersensitivity, including p1, p3, and i8, are of greatest interest because they contain sites that could potentially

![FIGURE 8. Phosphorimage analysis of CD21 fragment A DNase I HS PCR. Analyses were performed as previously described. Fragments that demonstrated identical hypersensitivity are shown as one plot. SD error bars are shown for only the first fragment listed for any given set. Data are the averages of at least three independent experiments.](http://www.jimmunol.org/DownloadedFrom/FIG8.jpg)
modulate the cell-specific expression of CD21. The lack of hypersensitivity in hyperacetylated T cells at p4 and i3 suggests that binding of NFs to these sites may not be required for CD21 expression. However, because hyperacetylated T cells express only about 50% of CD21 that B cells do, these binding events may serve a marginal enhancer function.

We have shown previously that inducing hyperacetylation in nonexpressing murine fibroblasts failed to induce CD21 expression, unlike the induction of T cell to express CD21 with the same stimulus (17). This finding suggested that B and T cells, which share the same lymphoid lineage, possess the necessary transcription factors to express CD21, while nonlymphoid cells do not. This represents one level of CD21 transcriptional control in which cell- or lineage-specific transcription factors are required for CD21 expression. In a parallel system, Romanow and colleagues recently demonstrated transcription-induced rearrangement of Ig genes in nonlymphoid cells following introduction of the V(D)J recombine

Another level of CD21 transcriptional control must involve regulating access to transcription factor binding sites by modulating chromatin structure. This could be accomplished by recruiting sequence-specific deacetylases to CD21 regulatory regions. A model system of this kind has been described for the mammalian corepressor mSin3A, which recruits histone deacetylases to regulatory elements in promoters (34–39). Recently, the B cell maturation regulator Blimp-1 and the lymphoid differentiation regulator Ikaros were both shown to repress transcription by recruiting deacetylases to promoters (40, 41). Blimp-1 and Ikaros are zinc finger NFs that bind specific DNA regulatory sites in distinct complexes. Ikaros has been shown to interact with mSin3 in vivo and in vitro, while Blimp-1 interacts directly with a histone deacetylase. Because we have shown that murine T cells express CD21 when their core histones are hyperacetylated (17), a key question to address in the future is whether any of the proteins demonstrated to bind to CD21 regulatory sites recruit or are themselves proteins that could alter the state of histone acetylation.

Finally, murine CD21 shares an expression profile similar to those of other B cell-specific proteins, including CD19, with which it forms a signal transduction complex, CD20 and CD22 (42). The MHC class II Aa gene also exhibits a similar expression pattern as CD21, in that it is expressed on B, but not T, cells (25). We used the cell-specific enhancer of MHC class II Aa as a positive control for DNase I hypersensitivity (Fig. 6). This enhancer has been previously shown to be hypersensitive in B, but not T, cells. Interestingly, when a hyperacetylated state was induced in murine T cells, the enhancer became hypersensitive. These data suggest a conserved regulatory mechanism for at least two and perhaps other genes with similar spatial and temporal expression patterns. The identification of specific transcription factors that modulate the chromatin structure in CD21, therefore, may illuminate a conserved function for those proteins in the regulation of other B cell-specific genes and provide insight into the role that transcriptional regulation plays in the biological function of the B cell.

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References


