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Regulation of the Pro-B-Cell-Specific Enhancer of the \textit{Id1} Gene
Involves the C/EBP Family of Proteins

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Received 18 September 1996/Returned for modification 22 October 1996/Accepted 7 November 1996

The \textit{Id1} protein acts as a negative regulator in early-B-cell differentiation by antagonizing the function of the basic helix-loop-helix (bHLH) transcription factors. Expression of the \textit{Id1} gene during B-cell development is governed at the transcriptional level primarily by a pro-B-cell-specific enhancer (PBE) located 3 kb downstream of the gene. We report here the identification of CAAT/enhancer binding protein β (C/EBPβ) as a component of the two major PBE-binding complexes (PBE1 and PBE2) found in pro-B cells by gel mobility shift assays. Formation of the PBECs is abolished when a classic C/EBP binding site is used as a competitor, and binding complexes similar to the PBECs are formed when the classic C/EBP site is used as a probe. We show that CHOP, a negative regulator of C/EBPs, specifically inhibits PBE binding in vitro and its enhancer activity in vivo. In pro-B cells, C/EBPβ binds to the PBE site not as apparent homodimers but possibly in association with at least one other polypeptide, which might determine the pro-B-cell-specific expression of the \textit{Id1} gene. Although isoforms of C/EBPβ are expressed in various B cells, they bind to DNA only in LyD9 and Ba/F3 pro-B cells. We show that CHOP is expressed in 70Z/3 and WEHI-231 cells. We also demonstrate that CHOP is associated with C/EBPβ in WEHI-231 cells, which may provide an additional mechanism to control the function of C/EBPβ and the expression of the \textit{Id1} gene.

\textit{Id1} is a member of the gene family that encodes negative regulators for the basic helix-loop-helix (bHLH) transcription factors (7, 36). Id proteins, which have the HLH domain but no basic region, dimerize with the E protein subclass of bHLH factors (E2A, E2-2, and HEB) and render them inactive for DNA binding or dimerization with other bHLH proteins. As a result, these transcription factors cannot activate downstream target genes. The opposing functions of the bHLH and Id proteins dictate the cellular programs for differentiation and proliferation of various cell types, i.e., the bHLH factors promote differentiation and suppress proliferation while Id proteins block the former and stimulate the latter (5, 18, 20, 23, 25, 33, 35).

The development of B lymphocytes can be divided into pro-B, pre-B, and mature B stages according to the rearrangement and expression of immunoglobulin (Ig) genes (17, 29). Pro-B cells are the earliest identified stage, during which the Ig genes are in the germ line configuration. When these cells undergo rearrangement and expression of the Ig heavy-chain locus, they become pre-B cells. Mature B cells have both the heavy-chain and light-chain loci rearranged and express surface IgM. The \textit{Id1} gene is thought to be expressed only in pro-B cells but not in later stages, while E2A gene products are present in all stages (19, 27, 36, 45). Constitutive expression of the \textit{Id1} gene in transgenic mice blocks the development of B cells at the pro-B stage, a phenotype similar to that caused by the null mutations of the E2A gene (3, 35, 45). This and several lines of evidence suggest a counter functional relationship between E proteins and Id in regulating B-cell development. In pro-B cells, Id proteins keep the existing E proteins inactive, while in subsequent stages, \textit{Id} expression is turned off to allow E proteins to stimulate the expression of downstream targets required for the differentiation of B cells. Therefore, expression of \textit{Id} genes must be precisely regulated in order for differentiation to proceed.

To elucidate the mechanisms regulating \textit{Id1} gene expression, we have previously shown that the \textit{Id1} gene is controlled at the transcriptional level by an enhancer element located approximately 3 kb downstream of the structural gene (31). This element can mediate the transcriptional activation of the luciferase reporter constructs driven by the promoter of the \textit{Id1} gene or by the minimal promoter of the \textit{c-fos} gene only in LyD9 and Ba/F3 pro-B-cell lines but not in PD31 pre-B and WEHI-231 mature-B-cell lines. (Although it is debatable whether LyD9 and Ba/F3 represent pro-B cells or earlier progenitors in hematopoietic development, for the sake of simplicity, we will refer to them as pro-B-cell lines.) This enhancer element was thus named PBE for its specific activity at the pro-B-cell stage. Specific PBE-binding complexes were also detected in nuclear extracts from pro-B cell lines, and they are referred to as PBE complexes (PBECS). To further understand B-cell stage-specific regulation of the \textit{Id1} gene, we screened a pro-B-cell cDNA expression library using concatemerized PBE sites as a probe and identified CAAT/enhancer binding protein β (C/EBPβ) as a positive clone. We subsequently provided several lines of in vitro and in vivo evidence to demonstrate that C/EBPβ is a component of PBECS not as homodimers but as heteromers with an unknown protein in pro-B cells. In WEHI-231 mature B cells, C/EBPβ is bound to CHOP, an inhibitor in the C/EBP family, and is thus inactivated. We suggest that the C/EBP family of proteins, by forming pro-B-cell-specific active complexes or mature-B-cell-specific inactive complexes, may play important roles in the regulation of the \textit{Id1} gene during B-cell development.

\textbf{MATERIALS AND METHODS}

\textbf{Cell lines, plasmids, and antibodies.} Mouse B-cell lines (LyD9, Ba/F3, PD31, 70Z/3, and WEHI-231) were grown in RPMI medium supplemented with 10% fetal bovine serum and 50 μM β-mercaptoethanol. The medium for LyD9 and

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Ba/F3 was also supplemented with 10% WEHI-3-conditioned medium as a source of interleukin-3. The luciferase reporter constructs were described previously (31). Constructs overexpressing C/EBPβ and CHOP in pcDNA 1 (In-vitrogen, San Diego, Calif.) were gifts from David Ron (30). C19 and Δ197 antibodies against C/EBPβ were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, Calif.). The antisera specific to the 31-kDa form (LAP) of C/EBPβ (NF66) and the monoclonal antibody to CHOP (37-9C8) were also gifts from David Ron (6, 30).

Library construction and screening. A Agt11 library was generated from random-primed cDNAs of pol(A)^+ RNA isolated from LyD9 pro-B cells by using the Stratagene (La Jolla, Calif.) cDNA Synthesis and Vector kits. A total of 9 × 10^7 plagues were screened with a 5-concentrated PBE probe according to the protocol described elsewhere (34, 40). The wild-type (WT) PBE sequence TCGAGGCTCCAGCCGAACTCAGG represents one unit of the concatemerized probe sequence. The PBE site is underlined and only the 5'-3' top strand is shown. The positive clone was purified, and the phage DNA was prepared. The insert was excised by digestion with EcoRI and was subcloned into the pBluescript II KS^+ (Stratagene) plasmid for sequencing.

EMSA, competition study, and supershift analysis. The electrophoretic mobility shift assay (EMSA) and competition studies were performed as described elsewhere (31). Typically, 10^6 pm of the 97-bp PBE-containing probe and 5 μg of crude nuclear extract were used in a binding reaction followed by analyses using 6% nondenaturing polyacrylamide gels and the Tri-glycine buffer unless otherwise indicated. For binding reactions with nuclear extract from COS-1 cells overexpressing C/EBPβ, 50 μg of the extract mixed with 5 μg of nondenatured COS-1 nuclear extract was used in each reaction. For supershift assays, 1 μg of the C19 or Δ198 antibody was added to the binding reaction mixture and incubated for 5 min before gel electrophoresis. In the EMSA involving CHOP, bacterially expressed glutathione S-transferase (GST)-CHOP or GST-CHOP LZ^- (gifts from David Ron) in the amounts equivalent to those used to disrupt the binding of C/EBPαs or C/EBPβ homodimers was incubated for 15 min with 5 μg of LyD9 crude nuclear extract prior to the binding reaction as previously described (30). The acute-phase response element (APRE)-M6 sequence, which contains a classic C/EBP binding site with the mutation (M6) that eliminated NF-κB binding to the APRE, was synthesized according to the published sequence (8). The sequence of oligonucleotide competitor (5'-3' top strand) of the mutated PBE is TCGAGGCTCCAGGCACTCAGG. The WT sequence is the same as that of the WT PBE oligonucleotide shown above. The PBE site is underlined and the mutated nucleotide is lowercase. Complexes bound to the APRE-M6 probe were resolved on 6% acrylamide gels in 0.5x Tris-borate EDTA (TBE) buffer.

Transfection and assay of luciferase activity. Approximately 5 × 10^6 LyD9 cells were transfected with 5 μg of reporter plasmid, 20 μg of CHOP, CHOP LZ^- , or carrier plasmid, and 1.5 μg of cytomegalovirus (CMV)-α-LacZ reporter as an internal control by the DEAE-dextran method (2). Cells were collected and assayed for luciferase and β-galactosidase activities after 2 days by using luciferase assay substrate (Promega, Madison, Wis.) and the Galacto-lite Plus kit (Tropix, Bedford, Mass.) respectively. Raw data of luciferase light units were first normalized with β-galactosidase activities. The relative activities were then calculated by dividing the normalized activities with that of the pBlUC construct, which represents basal activity. The data presented are from three separate transfection experiments.

Western blot (immunoblot) analysis and immunoprecipitation. Fifty micrograms of each nuclear extract was loaded on sodium dodecyl sulfate (SDS)-12%-polyacrylamide gels, and proteins were transferred to nitrocellulose membranes after electrophoresis. Western blots were incubated with a 1:1,000 dilution of the C19 anti-C/EBPβ antibody or a 1:5,000 dilution of anti-CHOP antibody. The immuno complexes were detected with a 1:2,500 dilution of the secondary horse-radish peroxidase-conjugated goat-anti-rabbit or anti-mouse IgG (Promega) and the ECL detection system (Amersham). For C/EBPβ-C/EBPβ immunoprecipitation, 4 × 10^7 LyD9 or WEHI-231 cells were lysed in 1.5 ml of radioimmunoprecipitation assay (RIPA) buffer by passing through a 21-gauge needle four to five times. Five micrograms of the C19 antibody was added to the lysate after proteinase K according to the standard protocol (2). To disrupt CHOP-C/EBPβ complexes, 4 × 10^7 WEHI-231 cells were lysed in 150 μl of RIPA buffer with 1% SDS and heated at 100°C for 5 min. The lysate was then brought up to 1.5 ml by addition of RIPA buffer without SDS followed by immunoprecipitation with the C19 antibody. The precipitates were resuspended in 60 μl of 2% sample buffer and heated at 100°C for 5 min. One-third of each sample was separated on an SDS-12% polyacrylamide gel and Western blotted with the anti-CHOP antibody.

RESULTS

C/EBPβ binds to PBE and is expressed in B cells. To identify the protein(s) that interacts with the PBE site, we constructed a λgt11 expression library from LyD9 pro-B-cell cDNAs and screened the library with a concatamer probe consisting of five copies of the PBE site. After 900,000 plagues had been screened, one positive clone was obtained. Sequence analysis of this clone revealed that it contained the cDNA encoding C/EBPβ. To determine the pattern of C/EBPβ expression in B cells, Western blot analysis with an anti-C/EBPβ antibody (C19) was performed. By alternative translation initiation, the mouse C/EBPβ transcript encodes three polypeptides with calculated molecular masses of 31, 29, or 16 kDa (10, 16). The two larger forms contain the transcriptional activation domain and are also termed liver-enriched transcriptional activator proteins (LAPs) (16). The smallest form lacks the activation domain and has a dominant negative effect on LAPs. It is therefore called liver-enriched transcriptional inhibitory protein (LIP) (16). Both the LAP (31-kDa) and LIP forms of C/EBPβ were detected in Ba/F3 and LyD9 pro-B cells but not in PD31 pre-B cells (Fig. 1). In 70Z/3 pre-B cells, the 31-kDa LAP is not present, but an antibody-reacting polypeptide that could correspond to the 29-kDa form of LAP is detected along with LAP. Interestingly, both LAP and LIP are found in WEHI-231 mature B cells, even though the PBECs are not detected in EMSAs. This raises the possibility that C/EBPβ may require a pro-B-cell-specific binding partner to form PBECs in pro-B cells or that a negative regulator may prevent C/EBPβ from binding to PBE in 70Z/3 and WEHI-231 cells.

PBEC formation can be inhibited by a classic C/EBP binding site. We have previously described a pro-B-cell-specific binding complex, now called PBE1C1, observed in EMSAs with a 97-bp PBE probe and 4% nondenaturing gels (31). By increasing the resolution of the polyacrylamide gels (6%), we subsequently discovered another faster-migrating pro-B-cell-specific complex and named it PBEC2 (Fig. 2). Since C/EBPβ was shown to bind to PBE by Southwestern screening of an Agt11 library and since the PBE site was not previously recognized by computer analysis as a C/EBP binding site (31), we attempted to verify whether C/EBPβ is a component of the PBECs found in pro-B cells. We first asked whether the PBECs share any DNA binding specificity with the C/EBPs. We performed a competition assay using oligonucleotides containing a classic C/EBP binding site (APRE-M6) as competitors in EMSA (8). The 97-bp probe bearing the PBE site was incubated with the LyD9 pro-B-cell nuclear extract in the absence or presence of the competitors (Fig. 2). The APRE-M6 oligonucleotides competed off PBE1C1 and PBEC2 nearly as efficiently as the WT PBE oligonucleotides, while a pair of PBE mutant oligonucleotides did not affect PBE binding at the same concentrations. We thus conclude that components of PBECs can also bind to the classic C/EBP binding site.

CHOP inhibits PBEC formation. To provide additional evidence that PBECs may contain C/EBPβ, we tested if the formation of PBECs can be abolished by CHOP, an inhibitor
of C/EBPα and -β (30). Bacterially expressed GST-CHOP, in the amounts comparable to those used to inhibit DNA binding by C/EBPβ homodimers, was added to the binding reaction mixtures in the EMSA (Fig. 3). Addition of CHOP at the concentration of 50 ng per reaction mixture begins to inhibit PBE binding activity and at 500 ng per reaction mixture completely abolishes both PBEC1 and PBEC2. In contrast, the CHOPLZ2 protein, which lacks the leucine zipper dimerization domain, cannot inhibit binding even at 500 ng per reaction mixture. These results further suggest that PBECs consist of one or more members of C/EBP family, which can dimerize with CHOP through the leucine zipper domain.

CHOP inhibits the enhancer activity of PBE. We then showed that the pro-B-cell-specific enhancer activity of PBE can be inhibited by CHOP in vivo. Cotransfection of the CHOP or CHOPLZ2 expression plasmid with the luciferase reporter gene controlled by PBE (pLUC/B1) was performed (Fig. 4). Compared to the expression of the reporter gene without the enhancer (pLUC), the PBE-containing sequence (pLUC/B1) activates the reporter by about 22-fold, while mutation at the PBE site (pLUC/B1s) abolishes 60% of this activity. CHOP but not CHOPLZ2 inhibits 90% of the PBE activity. Apparently, the inhibitory effect by CHOP is greater than the mutation at the PBE site itself. This is probably due to the presence of other potential C/EBP binding sites in the reporter plasmid. For example, two such sites are found 5 bp downstream and 50 bp upstream from the PBE. The presence of these putative C/EBP sites may explain why CHOP could further inhibit the remaining activity of the mutated PBE construct (pLUC/B1s). However, the inhibition by CHOP is specific to the PBE reporter because expression of the CMV-lacZ reporter construct used as an internal control was not affected by the presence of CHOP (data not shown). Taken together, these in vitro binding and in vivo transactivation data support the idea that members of the C/EBP family are involved in the binding of the PBE enhancer and the regulation of the Id1 gene.
PBECs contain C/EBPβ-, but the binding complexes are distinct from the C/EBPβ homodimer complexes. To determine whether C/EBPβ is a component of the PBECs, we performed an EMSA in the presence of antibodies against C/EBPβ. Both PBEC1 and PBEC2 in LyD9 cells were supershifted by two different antibodies against C/EBPβ, C19, and Δ198 (Fig. 5A). Apart from PBEC1 and PBEC2, LyD9 cells also display several minor complexes that cannot be supershifted by the anti-C/EBPβ antibodies and are present in varying quantities in non-pro-B cells such as WEHI-231 cells (Fig. 5A). Nuclear extract from WEHI-231 mature B cells did not show either PBEC1 or PBEC2; therefore, no supershifted complex was observed when the anti-C/EBPβ antibodies were added to the binding reaction mixtures. Since C/EBPβ proteins are known to bind to DNA as homodimers (16), we asked whether the PBECs and the C/EBPβ binding complexes comigrate in gel electrophoresis. C/EBPβ was overexpressed in COS-1 cells, and nuclear extract from these cells was prepared. In EMSAs, three PBE binding complexes were detected with this extract and could all be supershifted by the anti-C/EBPβ antibodies. These three complexes possibly represent the homo- and heterodimers of the 31- and 16-kDa forms of the C/EBPβ alternative translation products called LAP and LIP (16). The fastest-migrating complex would be to the LIP-LIP homodimer followed by the LAP-LIP heterodimer and the LAP/LAP homodimer. However, none of these complexes comigrates with either PBEC1 or PBEC2. Although PBEC2 and the potential LAP-LAP homodimer appeared to migrate similarly in this gel, we found them to be distinct upon prolonged electrophoresis (data not shown). Currently, we cannot rule out the possibility that different posttranslational modifications in LyD9 and COS-1 cells which would result in different migrating properties of C/EBPβ homodimers in EMSA may have occurred. However, our data strongly suggest that the PBECs are unlikely the simple homodimers of C/EBPβ.

Rather, they are probably heterodimers associated with a protein(s) that has not yet been identified and that may or may not be a member of the C/EBP family. Supershift experiments have ruled out C/EBPα or c-Fos as a dimerization partner of C/EBPβ in the PBECs (data not shown).

A minor band migrating faster than PBEC2 could also be supershifted by the anti-C/EBPβ antibodies. It probably represents another C/EBPβ complex either formed between its isoforms (e.g., LAP-LIP) or associated with other members of the C/EBP family (e.g., Ig/EBP).

What is the difference between PBEC1 and PBEC2? One likely explanation is that PBEC1 contains the 31-kDa LAP, while PBEC2 has the 16-kDa LIP. The supporting evidence came from an EMSA in the presence of a LAP-specific antibody, NF66 (Fig. 5B). Only PBEC1 but not PBEC2 was supershifted by the addition of this antibody. Incidentally, both the 31- and 16-kDa forms of C/EBPβ are expressed in LyD9 cells (Fig. 1). However, it is not clear whether PBEC1 and PBEC2 both activate transcription through PBE. This experiment also helps to argue that the PBECs may not contain the C/EBPβ homodimers. For example, the lack of LAP in PBEC2 would rule out the possibility that PBEC2 is similar to the LAP-LAP homodimer in the C/EBPβ-transfected COS-1 cells (Fig. 5A). The EMSA shown in Fig. 5B also displays a binding complex, which cannot be eliminated by CHOP and is present in only some of the preparations of nuclear extracts.

C/EBPβ in pro-B but not in mature B cells binds to the classic C/EBP binding site. Since C/EBPβ is also expressed in WEHI-231 mature B cells (Fig. 1), we were intrigued by the fact that it did not form complexes with PBE. One possibility is that C/EBPβ homodimers cannot bind to the PBE site efficiently and can do so only when overexpressed in COS-1 cells. We then asked whether C/EBPβ in WEHI-231 can bind to the classic C/EBP binding site. An EMSA was performed with a pair of the APRE-M6 oligonucleotides as a probe (Fig. 6). We
observed two binding complexes from the LyD9 nuclear extract that were supershifted by the two anti-C/EBP\(\beta\) antibodies. These complexes are probably equivalent to the PBECs found with the PBE probe. Again, none of these complexes comigrated with the C/EBP\(\beta\) dimer complexes in the C/EBP\(\beta\)-expressing COS-1 extract. In agreement with the finding with PBE as a probe, the WEHI-231 extract did not display any obvious binding complex that reacts with the anti-C/EBP\(\beta\) antibodies. These results led us to conclude that C/EBP\(\beta\) in WEHI-231 mature B cells is unable to bind to DNA even as a homodimer.

**CHOP is expressed and associated with C/EBP\(\beta\) in WEHI-231 cells.** One possible factor that can prevent C/EBP\(\beta\) from binding to DNA is CHOP (30). Therefore, we tested whether CHOP was expressed in any of the B-cell lines. Western blot analysis revealed that CHOP is expressed in the 70Z/3 pre-B and WEHI-231 mature-B-cell lines but not in Ba/F3, LyD9, and PD31 cell lines (Fig. 7A). The presence of CHOP in 70Z/3 and WEHI-231 cells may explain why C/EBP\(\beta\) can bind to DNA only in LyD9 and Ba/F3 cells even though 70Z/3 and WEHI-231 cells have equivalent levels of C/EBP\(\beta\) (Fig. 1). C/EBP\(\beta\) in 70Z/3 and WEHI-231 cells may be sequestered by CHOP and cannot homodimerize to bind to DNA.

To test this hypothesis, we then investigated whether CHOP and C/EBP\(\beta\) in WEHI-231 cells are physically associated with each other. The C19 antibody to C/EBP\(\beta\) was first used to immunoprecipitate extracts from WEHI-231 as well as LyD9 cells as a control. The immune complexes were then dissociated and analyzed by electrophoresis on an SDS-polyacrylamide gel electrophoresis gel. Subsequent Western blot analysis with an antibody against CHOP revealed that CHOP in the WEHI-231 extract was brought down by the antibodies against C/EBP\(\beta\) (Fig. 7B), indicating an interaction between C/EBP\(\beta\) and CHOP. To disrupt the interaction, the WEHI-231 cell extract was boiled for 5 min in RIPA buffer containing 0.1 M NaCl–1% SDS. After this treatment, a significantly smaller amount of CHOP was coimmunoprecipitated with C/EBP\(\beta\). CHOP is not expressed in LyD9 cells; therefore, it is not detected in the immunoprecipitated complex. We thus conclude that C/EBP\(\beta\) in WEHI-231 cells is associated with CHOP, which prevents C/EBP\(\beta\) from binding to either the classic C/EBP site or the PBE site.

**DISCUSSION**

The C/EBP family and its role in B-cell development. We have found previously that the PBE element located 3-kb downstream of the \(\text{id1}\) gene is central to regulation of the \(\text{id1}\) gene in pro-B-cell lines (31). Here, we report the identification of C/EBP\(\beta\) as one of the protein components that forms the pro-B-cell-specific PBECs, PBEC1 and PBEC2. There are several minor binding complexes in the pro-B-cell lines, but they are also present in WEHI-231 cells in quantities that vary from preparation to preparation. Some of these complexes cannot be competed off by the classic C/EBP oligonucleotides, while others cannot be inhibited by CHOP. Currently, we have not determined the identities and the functional significance of these minor complexes. However, the functional relevance of the C/EBP\(\beta\) containing PBEC1 and PBEC2 is emphasized by the fact that they represent the major PBEC existing only in \(\text{id1}\)-expressing pro-B cells.

C/EBP\(\beta\) (IL-6DBP and NF-IL6) belongs to a large protein family consisting of C/EBPs (1, 16, 26). These C/EBPs contain a conserved basic region and leucine zipper domain for DNA binding and dimerization (41). Members of this family act as either transcription activators or repressors in cell proliferation and differentiation (9, 39). They also mediate signal transduction (11, 37). Although the gene encoding C/EBP\(\beta\) does not contain any intron, alternative translation initiation of the C/EBP\(\beta\) transcript generates two major forms of the polypeptides, LAP and LIP (16). Because LIP does not possess the activation domain of LAP, it can bind to DNA but activates transcription poorly. Therefore, LIP may act as a dominant negative inhibitor to other C/EBPs under certain circumstances.

Ig/EBP and C/EBP\(\beta\) have been shown previously to be members of the C/EBP family that are expressed in the B-cell lineage (14). Ig/EBP is present throughout all stages of B-cell development, while C/EBP\(\beta\) is turned on later at mature B and plasma stages. We have shown here that C/EBP\(\beta\) is also expressed in two pro-B-cell lines, LyD9 and Ba/F3 (Fig. 1). While Ig/EBP is considered a general dominant negative inhibitor (12), the role of C/EBP\(\beta\) may be complicated by the presence

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**FIG. 6.** C/EBP\(\beta\) in LyD9 but not in WEHI-231 cells binds to the classic C/EBP site. The APRE-M6 probe and nuclear extracts from LyD9, WEHI-231, COS-1-expressing C/EBP\(\beta\), and COS-1 cells were employed in an EMSA. Antibodies to C/EBP\(\beta\) were added to the binding reaction mixtures as indicated. Pro-B-cell-specific complexes and supershift complexes associated with the antibodies are indicated by arrows.

**FIG. 7.** CHOP is expressed and associated with C/EBP\(\beta\) in WEHI-231 cells. (A) CHOP is expressed in 70Z/3 pre-B and WEHI-231 mature B cells but not in Ba/F3, LyD9, and PD31 cells. A Western blot analysis of nuclear extracts from various B-cell lines was performed with a monoclonal antibody to CHOP. (B) CHOP in WEHI-231 mature B cells is associated with C/EBP\(\beta\). Extracts from LyD9 and WEHI-231 cells were immunoprecipitated with the C19 anti-C/EBP\(\beta\) antisemur. The immune complex was resolved on an SDS-polyacrylamide gel electrophoresis gel, and CHOP was detected by Western blotting. In the lane marked heated, another sample of WEHI-231 extract was heated at 100°C for 5 min in 1% SDS prior to immunoprecipitation.
of LAP and LIP isomers, which act as an activator and a repressor, respectively. The classic C/EBP sites have been shown in cell culture to be important for the transcription of Ig \( V_{H} \) transcripts (13), as well as for the expression of the Ig \( \gamma \) (24, 44) and \( \epsilon \) (15) sterile transcripts involved in class switching at later stages of B-cell development. C/EBP\( \beta \) is also demonstrated in cell culture systems to activate interleukin-6 (IL-6) expression (1) and to participate in the IL-6 signaling pathway (11). However, the phenotype of C/EBP\( \beta \)-deficient mice suggests that C/EBP\( \beta \) acts as a negative factor in the lymphoid lineage (32). These mutant mice display an expansion of the B-cell compartment filled with B cells expressing surface IgG1, suggesting that class switching has taken place in the absence of C/EBP\( \beta \). They also have high rather than low levels of IL-6 in serum, as one would predict based on the data from cell culture studies. Although our results have shown that C/EBP\( \beta \) may be involved in the activation of \( Id1 \) gene expression in pro-B cells, lack of C/EBP\( \beta \) in mutant mice does not cause any defect in early-B-cell development. This is not surprising, because if reduction of \( Id1 \) expression occurs, it may be compensated by the presence of other Id proteins.

One exceptional member of the C/EBP family is CHOP, which has a nonconserved basic domain compared with other family members (30). CHOP can dimerize with C/EBPs or \( \beta \) and prevent them from binding to classic C/EBP binding sites. We have shown that CHOP is expressed and bound to C/EBP\( \beta \) in WEHI-231 mature B cells; however, CHOP is not present in the pro-B-cell lines. Interestingly, expression of CHOP has recently been shown to be induced by agents that cause endoplasmic reticulum (ER) stress by adversely affecting the function of the ER (28, 42). It would be interesting to determine if the increased burden of the ER to translocate and secrete Igs results of the ER (28, 42). It would be interesting to determine if the increased burden of the ER to translocate and secrete Igs

Association as well as the role of CHOP in regulating C/EBP\( \beta \) function in WEHI-231 mature B cells. Since the DNA-binding potential and transcriptional activating potential of the C/EBP family are known to be regulated by the signal transduction pathways of various growth factors and cytokines (22), it will be of profound interest to determine the effects of C/EBP\( \beta \) on Idl expression upon the posttranslational modification of C/EBP. Furthermore, the finding that \( Id1 \) expression can be inhibited by CHOP may suggest a possibility that induction of CHOP during growth arrest may lead to reduction of the expression of the growth-stimulating \( Id1 \) gene (4, 5, 25).

Although PBEC1 and PBEC2 are not found in other Id(1)-expressing cells, including myeloblasts, embryonic carcinoma cells, and myeloid precursor cells, the BSE site is indeed found to act as an enhancer there (30a, 31). It would be interesting to determine if the C/EBP family of transcription factors and protein X play any role in regulating \( Id1 \) expression in these cells.

ACKNOWLEDGMENTS

We are indebted to David Ron for numerous suggestions, valuable reagents, and critical reading of the manuscript. We thank Herbert Samuels and Harinder Singh for technical advice in library screening. We also thank Muktar Mahajan for critical reading of the manuscript. S.S. is a recipient of a scholarship from the Thai government. This work was supported by NIH grant AI33597 and the Lucille P. Markey Charitable Trust. X.-H.S. is a Cancer Research Institute Investigator and an Irma T. Hirschl Trust scholar.

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