Identification of *CD36* as the first gene dependent on the B-cell differentiation factor Oct-2

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The Oct-2 transcription factor is expressed predominantly in B lymphocytes and has been shown previously to be important for the terminal phase of B-cell differentiation in mice. A number of genes specifically expressed in B cells contain Oct-2-binding sites in their regulatory regions. However, the analysis of expression levels of these genes in Oct-2-deficient B cells revealed that they were unaffected. Hence, there were no genes known that critically depend on Oct-2 for their expression. To understand the molecular basis for the Oct-2 effect on B-cell development, we searched for Oct-2 target genes by subtractive cDNA cloning. We show here that expression of the murine *CD36* gene in B cells and macrophages requires a functional Oct-2 protein. Nuclear run-on experiments demonstrate that this gene is regulated transcriptionally by Oct-2. Moreover, *CD36* levels correlated with the levels of Oct-2 expression in several mouse B-cell and macrophage cell lines. Finally, compared to wild-type and heterozygous mice, *CD36* mRNA levels were markedly reduced in spleens and B-cell-enriched splenocyte fractions from *oct-2*^{-/-} mice. The data identify *CD36* as the first target gene critically dependent on Oct-2 for its expression. Because *CD36* expression is also dependent on Oct-2 in vivo, it is a candidate gene through which Oct-2 could affect B-cell differentiation.

[Key Words: B cell; *CD36*; Oct-2; subtractive cDNA cloning; target gene]

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Originally, the octamer motif was identified as a regulatory element critically involved in the B-cell-specific expression of immunoglobulin genes. However, octamer motifs were also identified in several other genes showing a ubiquitous expression pattern (for review, see Staudt and Lenardo 1991; Verrijzer and Van der Vliet 1993). Several transcription factors have been identified that interact functionally with this motif, which share significant homology in their DNA-binding domain, termed the POU domain (Herr et al. 1988). In B cells, two distinct octamer transcription factors are coexpressed. Oct-1, a ubiquitously expressed member of the family of octamer-binding proteins, and Oct-2, which is expressed predominantly in B lymphocytes but also can be detected in some T-cell and macrophage cell lines as well as in the nervous system (this study; Singh et al. 1986; Staudt et al. 1986, 1988; He et al. 1989; Hatzopoulos et al. 1990; Stoykova et al. 1992).

Oct-2 is expressed as a family of alternatively spliced mRNAs giving rise to several protein isoforms that all contain the POU domain and are therefore capable of binding to the octamer motif (Wirth et al. 1991). Oct-2 is expressed throughout B-cell differentiation and has been suggested to be crucial for the B-cell-restricted expres-

sion of immunoglobulin genes (Singh et al. 1986; Staudt et al. 1986, 1988; Dreyfus et al. 1987; Wirth et al. 1987; Müller et al. 1988; Junker et al. 1990; Schlissel et al. 1991). However, both Oct-1 and Oct-2 can activate transcription of octamer-dependent reporter constructs in vitro and in vivo, indicating significant functional overlap (LeBowitz et al. 1988; Pierani et al. 1990; Kemler et al. 1991; Luo et al. 1992; Annweiler et al. 1993). Nevertheless, recent data suggest that there are functional differences between the proteins. Whereas both Oct-1 and Oct-2 can activate an octamer element-driven reporter gene from a proximal promoter position, only Oct-2 can do so from a remote enhancer position (Annweiler et al. 1992; Pfisterer et al. 1994). This suggests the existence of genes that are activated by Oct-2 but not by Oct-1.

Additional genetic evidence for unique roles for Oct-2 in general development and in the B-cell differentiation pathway came from the analysis of *oct-2* null mutant (*oct-2*^{-/-}) mice generated by homologous recombination. These mice die within hours after birth for unknown reasons. In addition, they show severe defects in the terminal phase of differentiation from mature B cells to antibody-secreting plasma cells (Corcoran et al. 1993). A more detailed analysis of the molecular defect revealed that the G₀ to S-phase transition in the cell cycle with lipopolysaccharide (LPS) stimulation is severely im-

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paired in *oct-2*^{-/-} B cells (Corcoran and Karvelas 1994). These results suggested that developmentally relevant genes must exist that critically depend on Oct-2 for their expression. However, the *oct-2*^{-/-} phenotype also showed that Oct-2 is dispensable for the B-cell-specific expression of genes thought to be dependent on Oct-2, like the immunoglobulin genes (Corcoran et al. 1993). Analysis of the transcription rates of several other potential Oct-2 target genes in B cells from *oct2* null mutant animals failed to reveal any difference in expression when compared with that in normal B cells (Corcoran et al. 1993; Corcoran and Karvelas 1994). Thus far, no target genes for Oct-2 have been identified.

In an attempt to understand how Oct-2 could affect B-cell differentiation we searched for Oct-2 target genes by a subtractive and differential cDNA-cloning strategy based on an *oct-2*^{-/-} cell line that expresses a conditionally activatable Oct-2 protein. We show here that the murine gene for the membrane glycoprotein CD36 requires a functional Oct-2 protein for its expression in B cell lines and in the spleen of mice. Our results identify the murine CD36 gene as the first target gene critically dependent on Oct-2 in B lymphocytes. Whereas expression of CD36 has not been associated consistently with human B cells, we detected CD36 expression in a variety of murine B-cell lines as well as primary murine B cells. The known functions of CD36 make it a potential candidate through which Oct-2 could affect B-cell development.

Results

Identification of Oct-2-dependent target genes

To isolate Oct-2 target genes we established a subtractive and differential cDNA-cloning strategy (Fig. 1A) on the basis of an *oct-2*^{-/-} cell line. This cell line was generated from an Abelson virus-transformed fetal liver pre-B-cell line bearing a homozygous mutation of the endogenous *oct-2* gene (*abl1.1*) (Pfisterer et al. 1994). An estrogen-regulatable version of the Oct-2 protein, a chimera between Oct-2 and the hormone binding domain of the human estrogen receptor (Oct-2-ER), was expressed stably in these cells (*abl/Oct-2-ER*) (Pfisterer et al. 1994). Upon treatment of the cells with estrogen, Oct-2 becomes functionally activated. In normal B-cell development, Oct-2 is expressed continuously and we therefore reasoned that target genes for Oct-2 already would be expressed at early B-cell stages, although the prominent defect of the Oct-2 mutation is apparent only at a later stage of B-cell development (Corcoran et al. 1993; Corcoran and Karvelas 1994). We report here on two clones (clone 1.4 and 3.3) that we found by screening 2000 recombinant clones. They contain cDNA inserts of 2.1 and 2.7 kb, respectively. Sequencing revealed that the clones are derived from the same gene encoding the mouse homolog of the membrane glycoprotein CD36 (Endemann et al. 1993). Expression of both clones was not detectable in Northern blots using poly(A)⁺ RNA from untreated or estrogen-treated *abl1.1* cells and untreated *abl/Oct-2-ER*

Figure 1. Oct-2 target gene cloning. (A) Scheme of the differential cDNA-cloning strategy. Bacterial colonies of the subtractive cDNA library were screened with a subtracted cDNA probe generated by chemical cross-linking subtraction (CCLS). Positive colonies were rescreened in duplicate with cDNA probes from estrogen-treated *abl/Oct-2-ER* and *abl1.1* cells, respectively. Differentially hybridizing clones were tested for mRNA expression in Northern blot analysis. (B) Expression of clone 3.3 encoding a mouse homolog of CD36 is induced in *abl/Oct-2-ER* cells with estrogen treatment. Poly(A)⁺ RNA from *abl1.1* and *abl/Oct-2-ER* cells, treated with 1 μ M β -estradiol for 16 hr where indicated, was analyzed by Northern blot analysis. (C) CD36 expression is strongly induced by anti-estrogen hydroxy-tamoxifen. *abl/Oct-2-ER* cells were treated with the indicated concentrations of hydroxy-tamoxifen for 24 hr and poly(A)⁺ RNA was analyzed by Northern blotting.

cells (Fig. 1B). With estrogen treatment, however, two mRNA species of 2.3 and 3 kb are induced in the *abl/Oct-2-ER* cells (Fig. 1B). This suggests that CD36 expression is dependent on a functional Oct-2 protein. CD36 expression could also be induced by the anti-estrogen hydroxy-tamoxifen (Fig. 1C), indicating that activation of CD36 expression is not caused by the *trans*-activation domain in the ER hormone-binding domain (Webster et al. 1988).

Expression of murine CD36 correlates directly with Oct-2 levels in B cells and macrophages

From immunological studies with human and bovine cells and tissues, CD36 has been shown to be expressed on several cell types including macrophages, erythroid precursors, platelets, endothelial cells, and lactating mammary epithelial cells (for review, see Greenwalt et al. 1992). Although there is one report on a case of a human B-cell lymphoma that showed CD36 expression (Muroi et al. 1992), CD36 has not been reported to be expressed in B cells. Therefore, we analyzed CD36 expression in a variety of mouse B-cell lines. CD36 mRNA can be detected readily in murine B-cell lines corresponding to different developmental stages and the level of CD36 expression correlates with the expression level of Oct-2 (Fig. 2A). Expression is highest in WEHI-231 B cells containing high levels of Oct-2, intermediate in PD31 pre-B cells and S194 plasmacytoma cells, and lowest in 70Z/3 pre-B cells that express only marginal amounts of Oct-2. Electrophoretic mobility-shift assay (EMSA) experiments confirmed that the differences in amounts of *oct-2*-specific RNA were reflected at the level of functional Oct-2 protein in the various cell lines (data not shown). CD36 is also expressed in many other B-lymphoid cell lines such as the pre-B cell lines 300-19, 18-81, 204-1.8, 220-8, the B-cell lines A20-3 and WEHI-279, and the plasmacytoma cell line J558 (data not shown). In addition, the correlation between Oct-2 expression and CD36 expression also holds for the myeloid cell lines tested. Whereas RAW264.7 and RAW309Cr.1 express both Oct-2 and CD36, WEHI-3 lacks expression of both genes (Fig. 2A). NIH-3T3 fibroblasts and the BW5147 T-cell line also fail to express both Oct-2 and CD36 (Fig. 2A).

CD36 is also expressed in primary mouse B cells. Murine pre-B cells can be expanded from fetal liver on stroma cells in the presence of interleukin-7 (IL-7) (Rolink et al. 1991). These pre-B cells express readily detectable amounts of CD36, whereas no expression is detected in the stroma cells (Fig. 2B). CD36 is also expressed in spleen cells cultured in the presence of LPS for 3 days but not in concanavalin A (ConA) stimulated spleen cells (Fig. 2C). Whereas LPS treatment activates splenic B cells and induces terminal differentiation to plasma cells, ConA treatment results in T-cell activation; however, expression of CD36 is not restricted to hematopoietic cells. CD36 mRNA is expressed in several mouse organs, with high levels in kidney, spleen (Fig. 2E), lung, and heart (data not shown). Lower levels

were found in liver and thymus, and almost no CD36 mRNA was detectable in brain RNA (Fig. 2E).

The major defect in the differentiation of *oct-2^{-/-}* B cells occurs during the first 24 hr after stimulation of terminal differentiation by LPS (Corcoran et al. 1993; Corcoran and Karvelas 1994). Therefore, we analyzed the expression pattern of CD36 during this apparently critical phase of B-cell activation. CD36 is already expressed in unstimulated spleen cells (Fig. 2D). Immediately after stimulation, CD36 RNA levels decrease temporarily between 12 and 18 hr after stimulation, before the RNA reappears at an increased level at 24 hr (Fig. 2D). Expression levels appear slightly reduced again at 30 and 36 hr after stimulation and increased at 48 hr. As these cells show some synchronicity in their cell cycle progression, this finding might hint at a cell cycle regulation of CD36 during terminal B-cell differentiation. Additional experiments are required to confirm such a cell cycle dependence. Nevertheless, the observation that CD36 expression increases during the first 24 hr of LPS induction is in agreement with the result that Oct-2 is required for G₁ progression and proliferation during this induction process (Corcoran and Karvelas 1994).

Previous expression studies of CD36 had been performed primarily using detection of expressed protein by monoclonal antibodies on human cells. In these studies, CD36 was detected on platelets, monocytes, megakaryocytes, and endothelial cells but not typically on B-lineage cells. To determine whether murine CD36 is expressed as a stable protein on B cells we raised an anti-mouse CD36 peptide antiserum in rabbits. In Western immunoblot experiments with this antiserum we detected strong signals of 85–110 kD in WEHI-231 cell extracts (Fig. 3). Likewise, RAW264.7 macrophages also express CD36 protein, whereas the Oct-2-negative cell lines (WEHI-3 macrophages, NIH-3T3 fibroblasts, and BW5147 T cells) are negative for CD36 protein expression (Fig. 3). Most important, CD36 protein was only detectable in estrogen-treated *abl/Oct-2-ER* cells but not in the untreated cells (Fig. 3). Interestingly, the CD36 proteins from the *abl/Oct-2-ER* cells and the RAW264.7 cells appear somewhat smaller compared with those of the WEHI-231 B cells. CD36 has been shown to be a glycoprotein in several other species and differences in the glycosylation pattern have been observed in different cell lines (Greenwalt et al. 1992). Therefore, the higher molecular mass forms detected in WEHI-231 cells most likely represent differential protein modifications.

Oct-2 regulates CD36 directly at the transcriptional level

Oct-2 has been described as a transcriptional activator in B cells. Therefore, if CD36 is regulated directly by Oct-2, CD36 induction should occur at the transcriptional level. To test this hypothesis we performed the following experiments. First, we analyzed whether *de novo* protein synthesis would be necessary to induce CD36 expression. Therefore, induction of CD36 expression in *abl/Oct-2-ER* was performed in the presence of the protein

Figure 2. (A) CD36 and Oct-2 are coexpressed in different mouse cell lines. Northern blot analysis of poly(A)⁺ RNA from the indicated cell lines. The probes used are indicated at right. The small panel at left shows a longer exposure of the 70Z/3 lane. (B) CD36 is expressed in primary murine B cells. Total RNA from IL-7-dependent pre-B cells and the stroma cell line PA6 (control) were analyzed by Northern blot. (C) Northern blot analysis of CD36 expression in cultured spleen cells. Total cellular RNA from spleen cells cultured in the presence of either LPS (50 µg/ml) or ConA (5 µg/ml) and IL-2 for 72 hr was used. (D) CD36 expression in LPS-induced spleen cells. Nucleated spleen cells were cultured in the presence of LPS for the indicated time spans, and total RNA was analyzed. As glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression is also affected by LPS treatment (Annweiler et al. 1992), the ethidium bromide-stained RNA bands are shown at the bottom. (E) Analysis of CD36 mRNA expression in different mouse organs by RNase protection analysis using 10 µg of total RNA. Specifically protected bands are marked by arrows. DNA marker positions are shown at left.

synthesis inhibitor anisomycin. CD36 RNA expression could still be induced under these conditions (Fig. 4A). This result demonstrates that Oct-2 activates CD36 expression directly, without the essential requirement of intermediate gene products. However, the absolute level of induction was slightly lower than in the absence of anisomycin. This could be attributable to the fact that anisomycin also affects resynthesis of the Oct-2-ER fusion protein, whose levels will therefore decrease during the 8-hr induction period. Alternatively, transcriptional induction by Oct-2-ER might require an additional labile component whose synthesis is blocked by

anisomycin. Next, we performed the induction in the presence of the RNA polymerase inhibitor actinomycin D. No CD36 mRNA induction could be detected under these conditions, suggesting that the observed induction requires de novo RNA synthesis (Fig. 4B). Furthermore, the half-life of CD36 mRNA was not affected by estrogen treatment of the abl/Oct-2-ER cells (Fig. 4C). Finally, we directly measured the transcription rate of the CD36 gene by nuclear run-on analysis. Whereas the GAPDH control was only marginally affected by estrogen treatment, the transcription rate of the CD36 gene was clearly increased (Fig. 4D). Again transcriptional induc-

Figure 3. Western blot analysis of CD36 protein expression. Cell lysates (100 μ g of protein) from the indicated cell lines were separated on a 8% SDS–polyacrylamide gel, blotted, and probed with an anti-CD36 peptide anti-serum. The bar (right) marks the position of CD36 glycoproteins. Positions of protein molecular mass standards (in kilodaltons) are indicated at left.

tion was not significantly affected by the protein synthesis inhibitor anisomycin, arguing for a direct effect of the Oct-2 fusion protein (Fig. 4D). Quantitation of the signals in Figure 4D by PhosphorImager analysis revealed a fourfold stimulation of CD36 transcription by estrogen. Taken together, these results provide strong evidence that CD36 is induced transcriptionally with Oct-2–ER activation by estrogen.

CD36 expression is markedly reduced in the spleens of oct-2^{-/-} mice

It was important to determine whether CD36 expression would be affected in *oct-2*-deficient mice. These mice die within hours after birth and are severely impaired in the antigen-dependent phase of B-cell differentiation leading to antibody-secreting plasma cells (Corcoran et al. 1993; Corcoran and Karvelas 1994). CD36 expression was analyzed in spleens of 19-day-old embryos from the progeny of *oct-2*^{+/-} mice. Whereas CD36 mRNA was present in similar amounts in spleens from *oct-2*^{+/+} and *oct-2*^{+/-} embryos, it was only barely detectable in the spleen of the *oct-2*^{-/-} null mutant littermate (Fig. 5A). Whereas lymphocytes represent the major populations in adult spleen, spleens of day 19 embryos contain only minute numbers of B cells (<2%) but are an important site of myelopoiesis (Nossal and Pike 1973; Ohno et al. 1993). To strengthen the correlation between reduced CD36 expression and the B-cell compartment, we have enriched B220-positive cells from neonatal spleens by magnetic separation. Comparable amounts of B220-pos-

itive and B220/IgM double-positive cells were present in enriched fractions from heterozygous and Oct-2 mutant mice (Fig. 5B, top). However, whereas the CD36 signal was readily detectable by quantitative reverse transcriptase–polymerase chain reaction (RT–PCR) with cDNA from heterozygous cells, cells from Oct-2-deficient mice showed only a weak signal (Fig. 5B, bottom).

Our analysis of CD36 expression in various organs had indicated that CD36 is more widely expressed than Oct-2 (see Fig. 2E). One possible explanation for this could be that the expression of CD36 in non-hematopoietic tissues was attributable to circulating lymphocytes and/or macrophages expressing high levels of CD36. Alternatively, CD36 might be regulated differentially in different cell types and the strict Oct-2 dependence might be specific for lymphocytes and myeloid cells. We distinguished between these possibilities by analyzing RNA from whole embryos. No significant differences between wild-type or heterozygous embryos and *oct-2*-deficient embryos were found (data not shown). Thus, CD36 expression in different cell types is governed by distinct regulatory pathways and the Oct-2 requirement is specific for the hematopoietic lineages.

Discussion

Although Oct-2 had been known to be necessary late in B-cell development, namely in the differentiation of antibody-forming plasma cells, thus far no target genes for Oct-2 had been found. Genes that have been thought to be dependent on Oct-2 for their expression, like the immunoglobulin genes, B29 or CD20, showed no differences in their level of expression when *oct-2*^{-/-} B cells were compared with normal B cells (Corcoran et al. 1993; Feldhaus et al. 1993; Corcoran and Karvelas 1994). Thus, there had been no molecular targets known by which Oct-2 could affect B-cell development.

Using a subtractive and differential cDNA cloning approach we identified the cell-surface glycoprotein CD36 to be critically dependent on Oct-2 for its expression in B cells. Expression of CD36 mRNA and protein in the *abl*/Oct-2–ER cell line, which is null mutant for the endogenous *oct-2* genes and expresses only an estrogen-activatable Oct-2–ER fusion protein, was absolutely dependent on the activation of Oct-2–ER by estrogen. In addition, CD36 expression could be also induced by the anti-estrogen hydroxy-tamoxifen, which does not activate the *trans*-activation function in the estrogen receptor hormone-binding domain (Webster et al. 1988), indicating that CD36 mRNA induction is not caused by the ER portion of the Oct-2 fusion protein. CD36 expression could be demonstrated in a large number of murine B- and macrophage cell lines as well as in primary B cells and the expression level strictly correlated with the amounts of Oct-2 present in the respective cells. Induction of CD36 expression by estrogen in *abl*/Oct-2–ER cells occurs at the level of increased transcription and ultimately leads to the expression of CD36 protein in the induced cells. The requirement of CD36 expression for Oct-2 in hematopoietic cells in vivo is demonstrated by

Figure 4. Activation of CD36 by estrogen occurs at the level of transcription and in the presence of the protein synthesis inhibitor anisomycin. (A) Northern blot analysis of CD36 induction by estrogen in the presence of anisomycin. *abl/Oct-2-ER* cells were either left untreated or treated with β -estradiol ($1 \mu\text{M}$), anisomycin ($100 \mu\text{M}$), or both for the times indicated. (B) CD36 mRNA induction by estrogen in *abl/Oct-2-ER* cells is inhibited by the RNA polymerase inhibitor actinomycin D (Act.D). *abl/Oct-2-ER* cells were left either untreated or treated with $1 \mu\text{M}$ β -estradiol (E), $10 \mu\text{g/ml}$ of Act.D, or both (E + Act.D) for 8 hr, and poly(A)⁺ RNAs were analyzed by Northern blot analysis. (C) For determination of CD36 RNA stability, *abl/Oct-2-ER* cells were pretreated for 16 hr with β -estradiol, washed twice in PBS, and replated in the presence or absence of estrogen as indicated. Actinomycin D ($10 \mu\text{g/ml}$) was added and RNA was prepared at the time points indicated, and RNA was analyzed by Northern blot for CD36 (top) and GAPDH (bottom). Quantitation of the observed signals by PhosphorImager analysis revealed identical RNA decay rates in the presence and absence of β -estradiol. (D) Nuclear run-on analysis of CD36 transcription in *abl/Oct-2-ER* cells in the presence or absence of the protein synthesis inhibitor anisomycin. Cells were either left untreated (control) or treated for 4 hr with $1 \mu\text{M}$ β -estradiol, $100 \mu\text{M}$ anisomycin, or both β -estradiol and anisomycin, as indicated. Hybridization to GAPDH DNA served as internal control for the amount of labeled transcripts used in the hybridization reactions.

the strong reduction of CD36 expression in spleens and enriched B cells from *oct-2^{-/-}* mice.

The identification of CD36 as a target for Oct-2 in B cells was quite surprising, given that CD36 expression in humans is apparently not associated with B cells. Rather, in humans CD36 is expressed on myelomonocytic cells, platelets, megakaryocytes, and on endothelial cells (Greenwalt et al. 1992). However, there is one report on a CD36-expressing human B-cell lymphoma (Muroi et al. 1992), suggesting that CD36 might be also expressed in human B lymphocytes of certain stages. Alternatively, as most of the experiments with human cells have used monoclonal antibodies the possibility cannot be ruled out that CD36 protein on human B cells might not react with these antibodies. As CD36 is highly glycosylated in human cells it is conceivable that differential glycosylation of B-cell CD36 might interfere with detection by these antibodies. Cell-type-specific glycosylation has been described for CD36 proteins from different human and bovine tissues (Greenwalt et al. 1992 and references therein). Such differential glycosylation might also be

responsible for the different sizes of CD36 proteins in WEHI-231 and RAW264.7 cells reported here. Finally, we cannot rule out the possibility that mouse and human differ with respect to B-cell-specific CD36 expression. Such species-specific differences have been reported for other lymphocyte cell-surface markers. CD2 in humans is only expressed on T cells and not at all on B cells. In contrast, in the mouse CD2 is expressed both on T and B cells (Altevogt et al. 1989; Yagita et al. 1989).

The regulatory mechanisms that govern CD36 expression seem to differ in different cell types. Whereas CD36 RNA expression was strongly reduced in the spleen of homozygous *oct-2^{-/-}* mice, expression was unaffected in the other organs of these mice (the headless trunk without spleen was analyzed). This is in line with the tissue distribution of CD36, which is much wider than Oct-2 expression. Therefore, the strict dependence on Oct-2 seems to be confined to hematopoietic cells like B cells and macrophages.

CD36 is known as a membrane receptor for malaria-infected erythrocytes (Barnwell et al. 1989; Ockenhouse

Figure 5. Analysis of CD36 expression in spleen from normal and Oct-2-deficient mice. (A) Total RNA from spleens of day 19 mouse embryos was analyzed by Northern blot analysis (*bottom*). The genotype of the embryos is shown in a Southern blot analysis (*top*) using *KpnI*-restricted genomic DNA prepared from embryo heads and hybridized with an *oct-2* probe. The 6.2- and 4.4-kb bands indicate the mutant and wild-type alleles, respectively (Corcoran et al. 1993). (B) RT-PCR analysis of B220-enriched splenocytes from newborn *oct-2*^{-/-} and *oct-2*^{+/-} mice. FACS analysis of the cells used is depicted on *top* of the RT-PCR analysis. For FACS analysis, cells were stained with anti-B220 and anti-IgM antibodies; numbers indicate percentages of the cell populations. The enrichment for B220⁺ cells was ~15-fold. Increasing amounts of cDNA (0.5, 1.5, and 4.5 μ l) were used for PCR reactions with primers specific for CD36 or GAPDH.

et al. 1989; Oquendo et al. 1989), collagen (Tandon et al. 1989), and thrombospondin (Asch et al. 1987, 1992; Kieffer et al. 1988) and has been implicated in signal transduction via Src-like tyrosine kinases (Huang et al. 1991; Bull et al. 1994). In addition, CD36 has been described as a receptor and putative transporter for long-chain fatty acids (Abumrad et al. 1993; Harmon and Abumrad 1993). All of these known functions for CD36 are consistent with a role for CD36 in the differentiation pathway of B cells. Proliferation and differentiation of pre-B cells to B cells are dependent on their interaction with stroma cells in fetal liver and bone marrow (Kincaide et al. 1989; Strasser et al. 1989 and references therein). Also, during the late (antigen-dependent) phase of B-cell development resulting in plasma cells and memory cells, a step that is severely impaired in *oct-2*^{-/-} mice (Corcoran et al. 1993; Corcoran and Karvelas 1994), B cells have to be in close contact with other cells: the follicular dendritic cells (FDC) within the microenvironment of germinal centers in lymphoid organs (for

review, see Tew et al. 1990). The contact to FDCs is mandatory for the proliferation of germinal center B cells and their differentiation to plasma cells and memory cells (Kosco and Gray 1992; Tew et al. 1992; Lindhout et al. 1993). Thus, as a cell-surface receptor on B cells, CD36 could be involved in mediating cell-cell contacts important for B-cell development. In this respect it is interesting to note that *oct-2*^{-/-} B cells show a strongly reduced tendency for homotypic cell adhesion (L.M. Corcoran, unpubl.). By its association with tyrosine kinases (Huang et al. 1991; Bull et al. 1994), CD36 might transduce signals into the cell to trigger differentiation processes in response to such cell-cell contacts and/or yet unknown ligands. Intriguingly, the main defect in Oct-2-deficient B cells could be attributed to inefficient G_i progression and proliferation, a process that might require a costimulatory signal (Corcoran and Karvelas 1994). Finally, plasma cell differentiation is accompanied by the development of large amounts of endoplasmic reticulum making plasma cells to one of the most

efficiently secretory cell types known. Thereby, CD36 could be involved in providing fatty acids required for membrane synthesis and for energy supply by its role as a fatty acid receptor. In line with this is the strong expression of CD36 in tissues with high metabolic requirements for long-chain fatty acids like adipocytes, cardiac and microvascular endothelia, or lactating mammary epithelial cells [Knowles et al. 1984; Greenwalt et al. 1985; Greenwalt and Mather 1985; Abumrad et al. 1993; Harmon and Abumrad 1993].

Materials and methods

Construction of subtracted cDNA library and differential cDNA screening

cDNA was synthesized from poly(A)⁺ RNA of β -estradiol-treated (1 μ M for 16 hr) abl/Oct-2-ER cells, grown in Dulbeccos modified Eagle's medium (DMEM) without phenol red, using SuperScript II reverse transcriptase (GIBCO-BRL) and the primer restriction end adapter (PRE) 5'-CTAGAACGCGTTT-TTTTTTTTTT-3' (containing an *Xba*I site) as described [Coleclough and Erlitz 1985]. After removal of unincorporated primer molecules by alkaline agarose gel electrophoresis, ~250 ng of cDNA was subtracted with 10 μ g of poly(A)⁺ RNA from untreated abl1.1 cells using photoactivatable biotin and phenol extraction as described previously [Sive and St John 1988]. Upon dC-tailing, the cDNA was annealed to the plasmid Rc/CMV (Invitrogen Corp.), which had been dG-tailed at the *Bst*XI site and subsequently restricted with *Xba*I. Annealing, ligation (in the presence of the complementary PRE adapter 5'-AAAA-AAAACGCGTT-3'), and gap filling were done as described [Coleclough and Erlitz 1985]. The subtractive cDNA probe for the prescreening of bacterial colonies was generated by chemical cross-linking subtraction (CCLS) exactly as described [Hampson et al. 1992] using ~1 μ g of cDNA from estrogen-treated (for 16 hr) abl/Oct-2-ER cells, 20 μ g of poly(A)⁺ RNA from untreated abl1.1 cells and 2,5-diaziridinyl-1,4-benzochinone (DZQ) for cross-linking. The probe was then used to prescreen bacterial colonies that were picked on agar plates as small dashes (to distinguish unambiguously between colony-derived signals and background spots), grown at 37°C for 3–5 hr and then transferred to nylon membranes (Hybond-N, Amersham). Labeled probe (1 $\times$ 10⁶ cpm/ml) was used in hybridization reactions [Church and Gilbert 1984]. Positive colonies were rescreened with labeled cDNA probes derived from estrogen-treated abl/Oct-2-ER cells and abl1.1 cells, respectively. For cDNA probe preparation, cDNAs were synthesized as described above and 50 ng of single-stranded cDNA was labeled by random priming (Prime-it II, Stratagene) in the presence of 50 μ Ci of [α -³²P]dCTP.

RNA analyses

Four micrograms of poly(A)⁺ RNA or 10 μ g of total RNA was denatured and fractionated on 1% agarose gels containing 18% formaldehyde. The RNA was transferred to Hybond-N membrane and hybridized as described [Church and Gilbert 1984]. Total cellular RNA from organs and poly(A)⁺ RNA were prepared following standard protocols [Ausubel et al. 1989].

RNAse protection analysis was performed essentially as described in Wirth et al. [1991]. For probe synthesis a 198-bp *Ava*I-*Bgl*III fragment spanning the region between position 605 and 803 [Endemann et al. 1993] of the mouse CD36 cDNA was cloned into the *Eco*RV site of the plasmid Bluescript SK (Strat-

agene). Upon linearizing the template DNA by *Hind*III restriction, in vitro transcription using T3 RNA polymerase resulted in a probe of ~270 bp in size. CD36 mRNA protects 198 nucleotides.

For RT-PCR analysis, total cellular RNA was reverse transcribed for 1 hr at 41°C with 5 units of AMV-reverse transcriptase (Promega) in 20- μ l reactions containing 50 mM Tris-HCl (pH 8.3), 50 mM KCl, 10 mM MgCl₂, 10 mM DTT, 5 mM spermidine, 20 units of RNasin (Promega), and 100 ng of oligo[d(T)]₁₅ primer (Boehringer Mannheim). Samples were diluted with water to 200 μ l, and 0.5, 1.5, or 4.5 μ l was amplified in a reaction volume of 50 μ l containing 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris-HCl (pH 9.0), 250 μ M dNTPs, and 2.5 units of *Taq* polymerase (Pharmacia). Amplification parameters were as follows: 1 min at 94°C, 1 min at 55°C and 2 min at 72°C; 25 cycles were run for GAPDH and 30 cycles for CD36 amplifications, respectively.

GAPDH primers (13 pmoles each) were 5'-AGACAGCCG-CATCTTCTGTGC-3' (forward) and 5'-CTCCTGGAAGAT-GGTGATGG-3' (reverse).

CD36 primers (30 pmoles each) were 5'-GTGCTGATCCTT-TCAGAGTCTC-3' (forward) and 5'-CTGAGGAATGGATCT-TTGTAACC-3' (reverse).

Products of the PCR reactions were separated on agarose gels, blotted to Hybond-N membranes, and hybridized with labeled cDNA probes specific for the respective RNAs. Sizes of the amplified fragments were 720 bp (CD36) and 280 bp (GAPDH), respectively.

Nuclear run-on analysis

Preparation of nuclei and nuclear run-on analysis were performed as described [König et al. 1989]. CD36 transcripts were detected by hybridization to the 2.7-kb *Hind*III-*Xba*I cDNA insert of clone 3.3 immobilized on nitrocellulose filters.

Cell sorting and flow cytometry analysis

Magnetic separation was performed with the MiniMacs system using beads coated with anti-mouse B220 (CD45R) antibodies (Miltényi Biotec). For FACS analysis, cells were fixed with paraformaldehyde and stained with monoclonal antibodies against mouse B220 (CD45R) conjugated to R-phycoerythrin (GIBCO-BRL) and mouse IgM conjugated to fluorescein isothiocyanate (PharMingen). Flow cytometry analysis was performed with FACStar Plus (Becton Dickinson).

Antisera and Western blot analysis

For generation of anti-CD36 antisera, the peptide EGLHPNE-DEHRTYLDVEP corresponding to amino acids 358–375 of the mouse CD36 protein sequence [Endemann et al. 1993] was synthesized with a amino-terminal cysteine and coupled to maleimide-activated keyhole limpet hemocyanin (KLH) (Pierce). These KLH peptide conjugates were used for the immunization of rabbits.

Protein extracts for Western blot analysis were prepared by lysing cells in SDS lysis buffer [2% SDS, 62.5 mM Tris-HCl (pH 6.8), 10% glycerol] followed by sonification of the lysates to reduce viscosity. Lysate equivalents containing 100 μ g of protein were boiled in the presence of 5% β -mercapto-ethanol and separated on an 8% SDS-polyacrylamide gel. Western blot analysis was performed as described [Pfisterer et al. 1994] using 1:400 dilutions of rabbit antiserum and preimmune serum, respectively.

Cell culture

Cell lines were kept in DMEM/10% FCS (Glutamax GIBCO-BRL). Spleen cells were cultured in the presence of 50 µg/ml of LPS for the indicated time spans or treated for 72 hr with Con A (5 µg/ml) in medium supplemented with 5% supernatant from IL-2-overproducing cells (Karasuyama and Melchers 1988). Primary IL-7-dependent pre-B cells were cultured on PA6 stroma cells as described (Rolink et al. 1991).

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References

- Abumrad, N.A., M.R. El-Maghrabi, E.-Z. Amri, E. Lopez, and P.A. Grimaldi. 1993. Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. *J. Biol. Chem.* **268**: 17665–17668.
- Altevogt, P., M. Michaelis, and B. Kyewski. 1989. Identical forms of the CD2 antigen expressed by mouse T and B lymphocytes. *Eur. J. Immunol.* **19**: 1509–1512.
- Annweiler, A., U. Müller, and T. Wirth. 1992. Functional analysis of defined mutations in the immunoglobulin heavy-chain enhancer in transgenic mice. *Nucleic Acids Res.* **20**: 1503–1509.
- Annweiler, A., S. Zwilling, R.A. Hipskind, and T. Wirth. 1993. Analysis of transcriptional stimulation by recombinant Oct proteins in a cell free system. *J. Biol. Chem.* **268**: 2525–2534.
- Asch, A.S., J. Barnwell, R.L. Silverstein, and R.L. Nachman. 1987. Isolation of the thrombospondin membrane receptor. *J. Clin. Invest.* **79**: 1054–1061.
- Asch, A.S., S. Silbiger, E. Heimer, and R.L. Nachman. 1992. Thrombospondin sequence motif (CSVTCG) is responsible for CD36 binding. *Biochem. Biophys. Res. Commun.* **182**: 1208–1217.
- Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl. 1989. *Current protocols in molecular biology*, vol. 1. John Wiley & Sons, New York.
- Barnwell, J.W., A.S. Asch, R.L. Nachman, M. Yamaya, M. Aikawa, and P. Ingravallo. 1989. A human 88-kD membrane glycoprotein (CD36) functions in vitro as a receptor for a cytoadherence ligand on *Plasmodium falciparum*-infected erythrocytes. *J. Clin. Invest.* **84**: 765–772.
- Bull, H.A., P.M. Brickell, and P.M. Dowd. 1994. src-related protein tyrosine kinases are physically associated with the surface antigen CD36 in human dermal microvascular endothelial cells. *FEBS Lett.* **351**: 41–44.
- Church, G.M. and W. Gilbert. 1984. Genomic sequencing. *Proc. Natl. Acad. Sci.* **81**: 1991–1995.
- Coleclough, C. and F.L. Erlitz. 1985. Use of primer-restriction end adaptors in a novel cDNA cloning strategy. *Gene* **34**: 305–314.
- Corcoran, L.M. and M. Karvelas. 1994. Oct-2 is required early in T cell independent B cell activation for G1 progression and for proliferation. *Immunity* **1**: 635–645.
- Corcoran, L.M., M. Karvelas, G.J.V. Nossal, Z.-S. Ye, T. Jacks, and D. Baltimore. 1993. Oct-2, although not required for early B-cell development, is critical for later B-cell maturation and for postnatal survival. *Genes & Dev.* **7**: 570–582.
- Dreyfus, M., N. Doyen, and F. Rougeon. 1987. The conserved decanucleotide from the immunoglobulin heavy chain promoter induces a very high transcriptional activity in B-cells when introduced into an heterologous promoter. *EMBO J.* **6**: 1685–1690.
- Endemann, G., L.W. Stanton, K.S. Madden, C.M. Bryant, R.T. White, and A.A. Protter. 1993. CD36 is a receptor for oxidized low density lipoprotein. *J. Biol. Chem.* **268**: 11811–11816.
- Feldhaus, A.L., C.A. Klug, K.L. Arvin, and H. Singh. 1993. Targeted disruption of the Oct-2 locus in a B cell provides genetic evidence for two distinct cell type-specific pathways of octamer element-mediated gene activation. *EMBO J.* **12**: 2763–2772.
- Greenwalt, D.E., and I.H. Mather. 1985. Characterization of an apically derived epithelial membrane glycoprotein from bovine milk, which is expressed in capillary endothelia in diverse tissues. *J. Cell. Biol.* **100**: 397–408.
- Greenwalt, D.E., V.G. Johnson, and I.H. Mather. 1985. Specific antibodies to PAS IV, a glycoprotein of bovine milk-fat-globule membrane, bind to a similar protein in cardiac endothelial cells and epithelial cells of lung bronchioles. *Biochem. J.* **228**: 233–240.
- Greenwalt, D.E., R.H. Lipsky, C.F. Ockenhouse, H. Ikeda, N.N. Tandon, and G.A. Jamieson. 1992. Membrane glycoprotein CD36: A review of its role in adherence, signal transduction, and transfusion medicine. *Blood* **80**: 1105–1115.
- Hampson, I.N., L. Pope, G.J. Cowling, and T.M. Dexter. 1992. Chemical cross linking subtraction (CCLS): A new method for the generation of subtractive hybridization probes. *Nucleic Acids Res.* **20**: 2899.
- Harmon, C.M. and N.A. Abumrad. 1993. Binding of sulfosuccinimidyl fatty acids to adipocyte membrane proteins: Isolation and amino-terminal sequence of an 88-kD protein implicated in transport of long-chain fatty acids. *J. Membrane Biol.* **133**: 43–49.
- Hatzopoulos, A.K., A.S. Stoykova, J.R. Erselius, M. Goulding, T. Neuman, and P. Gruss. 1990. Structure and expression of the mouse Oct-2a and Oct-2b, two differentially spliced products of the same gene. *Development* **109**: 349–362.
- He, X., M.N. Treacy, D.M. Simmons, H.A. Ingraham, L.W. Swanson, and M.G. Rosenfeld. 1989. Expression of a large family of POU-domain regulatory genes in mammalian brain development. *Nature* **340**: 35–42.
- Herr, W., R.A. Sturm, R.G. Clerc, L.M. Corcoran, D. Baltimore, P.A. Sharp, H.A. Ingraham, M.G. Rosenfeld, M. Finney, G. Ruvkun, and H.R. Horvitz. 1988. The POU domain: A large conserved region in the mammalian *pit-1*, *oct-1*, *oct-2*, and *Caenorhabditis elegans unc-86* gene products. *Genes & Dev.* **2**: 1513–1516.
- Huang, M.-M., J.B. Bolen, J.W. Barnwell, S.J. Shattil, and J.S. Brugge. 1991. Membrane glycoprotein IV (CD36) is physically associated with the *fyn*, *lyn*, and *yes* protein-tyrosine kinases in human platelets. *Proc. Natl. Acad. Sci.* **88**: 7844–7848.
- Junker, S., S. Pedersen, E. Schreiber, and P. Matthias. 1990. Extinction of an immunoglobulin κ promoter in cell hybrids is mediated by the octamer motif and correlates with suppression of Oct-2 expression. *Cell* **61**: 467–474.
- Karasuyama, H. and F. Melchers. 1988. Establishment of mouse

- cell lines which constitutively secrete large quantities of interleukin 2, 3, 4, or 5 using modified cDNA expression vectors. *Eur. J. Immunol.* **18**: 97–104.
- Kemler, I., E. Bucher, K. Seipel, M.M. Müller-Immerglück, and W. Schaffner. 1991. Promoters with the octamer DNA motif (ATGCAAAT) can be ubiquitous or cell type-specific depending on binding affinity of the octamer site and Oct-factor concentration. *Nucleic Acids Res.* **19**: 237–242.
- Kieffer, N., A.T. Nurden, M. Hasitz, M. Titeux, and J. Breton-Gorius. 1988. Identification of platelet membrane thrombospondin binding molecules using an anti-thrombospondin antibody. *Biochim. Biophys. Acta* **967**: 408–415.
- Kincade, P.W., G. Lee, C.E. Pietrangeli, S.-I. Hayashi, and J.M. Gimble. 1989. Cells and molecules that regulate B lymphopoiesis in bone marrow. *Annu. Rev. Immunol.* **7**: 111–143.
- Knowles, D.M., B. Tolidjian, C. Marboe, V. D'Agati, M. Grimes, and L. Chess. 1984. Monoclonal anti-human monocyte antibodies OKM1 and OKM5 possess distinct tissue distributions including differential reactivity with vascular endothelium. *J. Immunol.* **132**: 2170–2173.
- König, H., H. Ponta, U. Rahmsdorf, M. Büscher, A. Schönthal, H.J. Rahmsdorf, and P. Herrlich. 1989. Autoregulation of fos: The dyad symmetry element as the major target of repression. *EMBO J.* **8**: 2559–2566.
- Kosco, M.H. and D. Gray. 1992. Signals involved in germinal center reactions. *Immunol. Rev.* **126**: 63–76.
- LeBowitz, J.H., T. Kobayashi, L. Staudt, D. Baltimore, and P.A. Sharp. 1988. Octamer-binding proteins from B or HeLa cells stimulate transcription of the immunoglobulin heavy-chain promoter *in vitro*. *Genes & Dev.* **2**: 1227–1237.
- Lindhout, E., M.L.C.M. Mevissen, J. Kwekkeboom, J.M. Tager, and C. De Groot. 1993. Direct evidence that human follicular dendritic cells (FDC) rescue germinal centre B cells from death by apoptosis. *Clin. Exp. Immunol.* **91**: 330–336.
- Luo, Y., H. Fujii, T. Gerster, and R.G. Roeder. 1992. A novel B cell-derived coactivator potentiates the activation of immunoglobulin promoters by octamer-binding transcription factors. *Cell* **71**: 231–241.
- Müller, M.M., S. Ruppert, W. Schaffner, and P. Matthias. 1988. A cloned octamer transcription factor stimulates transcription from lymphoid-specific promoters in non-B cells. *Nature* **336**: 544–551.
- Muroi, K., K. Toya, T. Suzuki, T. Suda, S. Sakamoto, and Y. Miura. 1992. Expression of CD11B, CD14 and CD36 antigens by B-cell lymphoma. *Br. J. Haematol.* **80**: 126–127.
- Nossal, G. and B. Pike. 1973. Studies on the differentiation of B lymphocytes in the mouse. *Immunology* **25**: 33–45.
- Ockenhouse, C.F., N.N. Tandon, C. Magowan, G.A. Jamieson, and J.D. Chulay. 1989. Identification of a platelet membrane glycoprotein as a *Falciparum malaria* sequestration receptor. *Science* **243**: 1469–1471.
- Ohno, H., M. Ogawa, S. Nishikawa, S.-i. Hayashi, T. Kunisada, and S.-i. Nishikawa. 1993. Conditions required for myelopoiesis in murine spleen. *Immunol. Lett.* **35**: 197–204.
- Oquendo, P., E. Hundt, J. Lawler, and B. Seed. 1989. CD36 directly mediates cytoadherence of *Plasmodium falciparum* parasitized erythrocytes. *Cell* **58**: 95–101.
- Pfisterer, P., A. Annweiler, C. Ullmer, L. Corcoran, and T. Wirth. 1994. Differential transactivation potential of Oct1 and Oct-2 is determined by additional B cell-specific activities. *EMBO J.* **13**: 1654–1663.
- Pierani, A., A. Heguy, H. Fujii, and R.G. Roeder. 1990. Activation of octamer-containing promoters by either octamer-binding transcription factor 1 (OTF-1) or OTF-2 and requirement of an additional B-cell-specific component for optimal transcription of immunoglobulin promoters. *Mol. Cell. Biol.* **10**: 6204–6215.
- Rolink, A., A. Kudo, H. Karasuyama, Y. Kikuchi, and F. Melchers. 1991. Long-term proliferating early pre B cell lines and clones with the potential to develop to surface Ig-positive, mitogen reactive B cells *in vitro* and *in vivo*. *EMBO J.* **10**: 327–336.
- Schlissel, M., A. Voronova, and D. Baltimore. 1991. Helix-loop-helix transcription factor E47 activates germ-line immunoglobulin heavy-chain gene transcription and rearrangement in a pre-T-cell line. *Genes & Dev.* **5**: 1367–1376.
- Singh, H., R. Sen, D. Baltimore, and P.A. Sharp. 1986. A nuclear factor that binds to a conserved sequence motif in transcriptional control elements of immunoglobulin genes. *Nature* **319**: 154–158.
- Sive, H.L. and T. St. John. 1988. A simple subtractive hybridization technique employing photoactivatable biotin and phenol extraction. *Nucleic Acids Res.* **16**: 10937.
- Staudt, L.M. and M.J. Lenardo. 1991. Immunoglobulin gene transcription. *Annu. Rev. Immunol.* **9**: 373–398.
- Staudt, L.M., H. Singh, R. Sen, T. Wirth, P.A. Sharp, and D. Baltimore. 1986. A lymphoid-specific protein binding to the octamer motif of immunoglobulin genes. *Nature* **323**: 640–643.
- Staudt, L.M., R.G. Clerc, H. Singh, J.H. LeBowitz, P.A. Sharp, and D. Baltimore. 1988. Cloning of a lymphoid-specific cDNA encoding a protein binding the regulatory octamer DNA motif. *Science* **241**: 577–580.
- Stoykova, A.S., S. Sterrer, J.R. Erselius, A.K. Hatzopoulos, and P. Gruss. 1992. Mini-Oct and Oct-2c: Two novel, functionally diverse murine Oct-2 gene products are differentially expressed in the CNS. *Neuron* **8**: 541–558.
- Strasser, A., A. Rolink, and F. Melchers. 1989. One synchronous wave of B cell development in mouse fetal liver changes at day 16 of gestation from dependence to independence of stromal cell environment. *J. Exp. Med.* **170**: 1973–1986.
- Tandon, N.N., U. Kralisz, and G.A. Jamieson. 1989. Identification of glycoprotein IV (CD36) as a primary receptor for platelet-collagen adhesion. *J. Biol. Chem.* **264**: 7576–7583.
- Tew, J.G., M.H. Kosco, G.F. Burton, and A.K. Szakal. 1990. Follicular dendritic cells as accessory cells. *Immunol. Rev.* **117**: 185–211.
- Tew, J.G., R.M. DiLosa, G.F. Burton, H.M. Kosco, L.I. Kupp, A. Masuda, and A.K. Szakal. 1992. Germinal centers and antibody production in bone marrow. *Immunol. Rev.* **126**: 99–112.
- Verrijzer, C.P. and P.C. Van der Vliet. 1993. POU domain transcription factors. *Biochim. Biophys. Acta* **1173**: 1–21.
- Webster, N.J.G., S. Green, J. Rui Jin, and P. Chambon. 1988. The hormone-binding domains of the estrogen and glucocorticoid receptors contain an inducible transactivation function. *Cell* **54**: 199–207.
- Wirth, T., L. Staudt, and D. Baltimore. 1987. An octamer oligonucleotide upstream of a TATA motif is sufficient for lymphoid-specific promoter activity. *Nature* **329**: 174–178.
- Wirth, T., A. Priess, A. Annweiler, S. Zwilling, and B. Oeler. 1991. Multiple Oct-2 isoforms are generated by alternative splicing. *Nucleic Acids Res.* **19**: 43–51.
- Yagita, H., T. Nakamura, H. Karasuyama, and K. Okumura. 1989. Monoclonal antibodies specific for murine CD2 reveal its presence on B as well as T cells. *Proc. Natl. Acad. Sci.* **86**: 645–649.

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