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Immunology

Three distinct signals can induce class II gene expression in a murine pre-B-cell line

(B-cell-stimulatory factor 1/*trans*-acting factor/gene regulation)

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ABSTRACT **Expression of class II genes of the major histocompatibility complex (MHC) has been studied in an Abelson-murine-leukemia-virus-transformed pre-B-cell line, R8, and its class II molecule (Ia)-negative variant, R8205. These variant cells contained barely detectable levels of RNA specific for all class II genes, including the nonpolymorphic invariant chain gene (*Ii*), and did not express cell surface Ia. Fusion of this murine Ia-negative cell line to the human Ia-positive Raji cell produced an interspecies hybridoma that expressed the murine Ia. These data are further evidence for the existence of a *trans*-acting factor(s) that can regulate class II gene expression. Furthermore, the T-cell-derived lymphokine B-cell-stimulatory factor 1 (BSF-1) induced expression of class II genes in the R8205 cells. Exposure of R8205 cells to an antibody that has been shown to mimic BSF-1 activity on normal B cells also resulted in expression of class II genes. These data demonstrate that three distinct signals—a lymphokine, an alloantibody binding to membrane structures, and an interspecies *trans*-acting factor—can induce expression of class II genes.**

The magnitude of the immune response is controlled, in part, by the level of expression of class II molecules on the surface of antigen-presenting cells (1). The synthesis and expression of class II antigens on such cells can be modulated by a number of different stimuli. Several groups have reported that class II gene expression is regulated by the T-cell-derived lymphokine interferon γ (IFN-γ) (2, 3). More recently, another lymphokine secreted by T cells, B-cell-stimulatory factor 1 (BSF-1), initially described as a required costimulant with low concentrations of anti-IgM antibodies for DNA synthesis in resting B cells, has been shown to increase the expression of Ia molecules (class II locus products) on small resting B cells (4, 5). B-cell expression of Ia molecules can also be increased, both *in vivo* and *in vitro*, by cross-linking B-cell receptors with anti-Ig antibodies (6). The existence of *trans*-acting factor(s) regulating Ia expression has been demonstrated by the expression of human Ia genes in hybrids between Ia-negative human regulatory mutants and Ia-positive mouse B-cell lines (7–9). Previously reported Ia-negative cell lines, however, including SCID cells (10), did contain transcripts for the nonpolymorphic invariant chain gene (*Ii*) and were not inducible by lymphokine.

We have also approached the problem of class II gene regulation by studying the expression of such genes and their products in an Ia-negative variant cell line. This line, R8205, is derived from an Abelson-virus-transformed mouse pre-B cell and contains barely detectable class II specific messenger RNA (mRNA), including Ii mRNA. We studied the effects of fusion of the R8205 Ia-negative murine pre-B-cell line to the Ia-positive human Raji cell line on murine class II gene expression. Furthermore, we investigated the effects of the lymphokine BSF-1 on the expression of class II genes in this cell line. We also asked whether an antibody directed against the B-cell-specific surface structure Lyb-2, which has been found to mimic BSF-1 activity in normal B cells (E. M. Rabin, J. J. Mond, J.O., and W.E.P., unpublished results), could induce Ia expression. We report here, in agreement with others, that an interspecies *trans*-acting factor(s) could restore class II gene expression in the R8205 cell line. In contrast to other reports (10), the T-cell-derived lymphokine BSF-1 induced expression of these genes as well.

MATERIALS AND METHODS

Cells. R8 is a heterozygous $H\text{-}2^b \times H\text{-}2^d$ surface-Ig-negative pre-B-cell line transformed by Abelson murine leukemia virus (11). The Ia-negative variant R8205 was selected by immunoselection with a K^b class I-specific monoclonal antibody (mAb); it has a serologically altered K^b molecule (S.G.G., R. A. Zeff, T. V. Rajan, and S.G.N., unpublished results). Raji is an Ia-bearing human B lymphoma cell line (class II phenotype: DR3,w6; DQ1; NG2).

All cells were maintained in RPMI 1640 medium (Biofluids, Rockville, MD) supplemented with 5% fetal calf serum, 10 μM Hepes, penicillin at 100 units/ml, 100 μM 2-mercaptoethanol, and 2 mM L-glutamine.

BSF-1. Purified BSF-1 was prepared as previously described (12).

Antibodies. Anti-Lyb-2.2 antiserum was a gift of F. W. Shen (Sloan–Kettering, New York). Lyb-2.2 is the allelic form of Lyb-2 expressed by the BALB/c and C57BL/6 mouse strains from which the R8 cell is derived. A mAb against the alternative allelic form of Lyb-2 (Lyb-2.1) was a gift of H. Morse (National Institutes of Health). Both reagents were used in culture at a final dilution of 1:100. Hybridomas secreting anti-I-$A^{b,d}$ (34-5-3) and anti-I-E^d (14-4-4) were gifts of David Sachs (National Institutes of Health) and were used to prepare ascitic fluid containing these mAbs.

Flow Cytometric Analysis. Flow cytometric analysis was performed by using a Becton Dickinson FACS analyzer as previously described. The specificity control for staining by anti-Ia mAb followed by fluorescein-conjugated goat anti-

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Abbreviations: BSF-1, B-cell-stimulatory factor 1; Ia molecule, class II locus product; Ii, invariant chain; kb, kilobase(s); mAb, monoclonal antibody; MHC, major histocompatibility complex; IFN-γ, interferon γ.

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body to mouse Ig was the fluorescence histogram obtained with the latter reagent alone.

RNA Analysis. Total cellular RNA was isolated by a method using guanidine isothiocyanate (13). Samples of RNA (10–15 μg) were electrophoresed on 1.3% agarose gels containing formaldehyde and transferred to nitrocellulose as described (14). DNA and RNA blots were hybridized to probes specific for the Aα, Eβ, Aβ, invariant chain and class I genes. A 1.3-kilobase (kb) *Hin*dIII DNA fragment derived from a recently cloned A^b_α gene (15) and a 2.1-kb *Bam*HI fragment derived from the A^k_β gene (16) were used as probes to detect Aα and Aβ sequences, respectively. A 2.0-kb *Eco*RI fragment derived from the 5′ end of the *Eβ* gene (kindly provided by L. Hood) (17) was used as probe to detect *Eβ* sequences. The 0.9-kb cDNA invariant chain (Ii) probe was the gift of Paul Singer (University of California, Los Angeles) (18). The class I probe was the gift of J. Seidman (Harvard Medical School) (19). To control for amount of RNA loaded in gel slots, we used the A50 DNA, a cDNA clone containing sequences for a constitutively expressed "housekeeping" mRNA of unknown identity (20).

RESULTS

Analysis of the Abelson-Virus-Transformed Pre-B-Cell Line R8 and Its Ia-Negative Variant R8205. The R8 cell is an Abelson-virus-transformed BALB/c × C57BL/6 (H-2^b × H-2^d) surface Ig-negative pre-B-cell line (11). The Ia-negative variant of R8, R8205, which was obtained in the course of immunoselection with a K^b class I-specific mAb, has a serologically altered K^b molecule (S.G.G. and S.G.N., unpublished data). In addition to the subtle alteration in the class I K^b molecule, the R8205 cell has lost all cell surface expression of class II molecules. Thus, in contrast to the parental line R8, the R8205 cell displays no reactivity by flow cytometric analysis with mAbs specific for the I-$A^{b,d}$ and I-E molecules (Fig. 1 *Upper*). As shown in Fig. 1 *Lower*, mRNAs for the *Aα*, *Aβ*, *Eβ*, and *Ii* genes were not detected by blot hybridization analysis. *Ii* is a nonpolymorphic class II gene. The R8205 cell thus differs from the null variants derived by others (21, 22), which do contain transcripts for the Ii chain. Only when a purified preparation of poly(A)$^+$ RNA was examined was a faint signal detected for class II gene transcripts (not shown). Southern blot hybridization analysis revealed that the class II genes were present and apparently normal in the R8205 cell (not shown). The levels of mRNAs for the class I genes in the R8205 cell were not different from those in the parental line (Fig. 1 *Lower*). R8205 is a stable cell line, and we observed no revertants over a 9-month period.

Activation of Murine Ia Expression by Somatic Cell Hybridization. A hypoxanthine/guanine phosphoribosyltransferase-deficient variant of R8205, R8205.C6, was derived by selection with 6-thioguanine after mutagenesis of the R8205 line with ethyl methanesulfonate (23). These cells fail to survive in hypoxanthine/aminopterin/thymidine (HAT) selective medium. Somatic cell hybrids between R8205.C6 and the human Ia-positive B lymphoma Raji were obtained by fusion utilizing M_r 4000 polyethylene glycol as described (24). Selection of hybrids was carried out in HAT medium containing 1 μM ouabain (25). Cells growing in selective medium were analyzed by flow cytometry for expression of Ia molecules. Two out of four hybrids screened were positive for murine class II gene expression. Fig. 2 *Left* shows the flow cytometric profile of one of these hybrids that expresses significant amounts of murine I-$A^{b,d}$ as well as the human HLA class I determinants, providing evidence for the presence of both human and murine chromosomes in the hybrid cells. Blot hybridization analysis revealed the presence of murine class II-specific mRNA in the hybrid cells (Fig. 2 *Right*). A probe specific for the murine *Eβ* gene was used: the

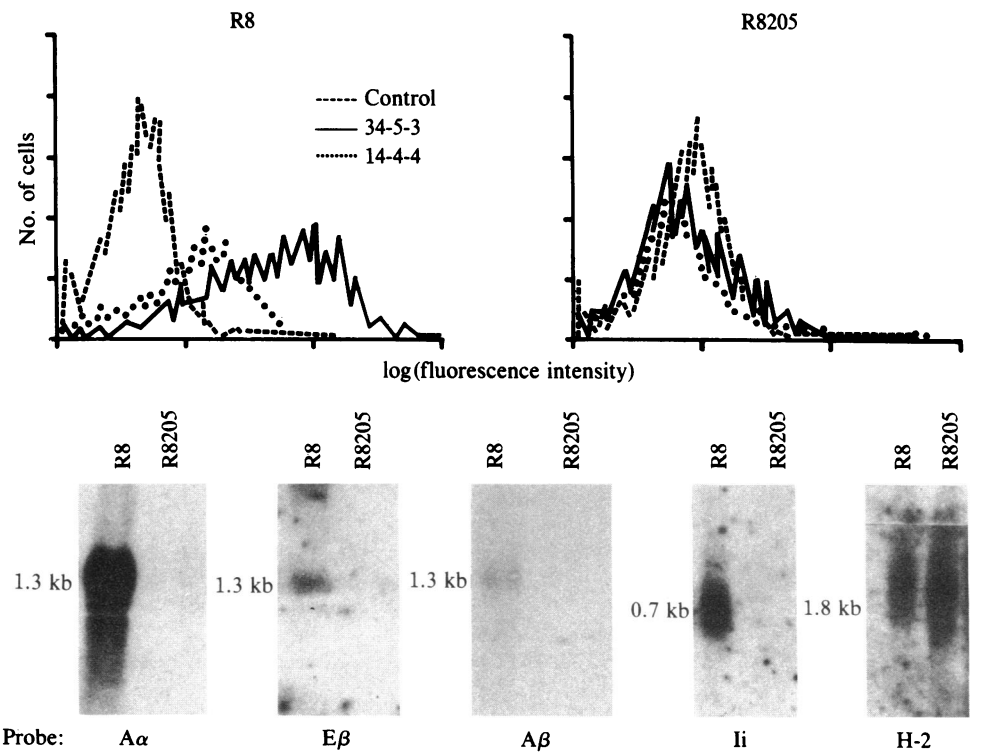

FIG. 1. Analysis of the Abelson-virus-transformed pre-B-cell line R8 and its Ia-negative variant R8205. (*Upper*) Flow cytometry analysis of expression of $Ia^{b,d}$ molecules on the surfaces of the R8 and R8205 cells. Reactivities with the anti-I-$A^{b,d}$ mAb 34-5-3 and the anti-I-E^d mAb 14-4-4 are shown. Control is the fluorescence histogram obtained with the fluorescein-conjugated goat anti-mouse Ig reagent alone. (*Lower*) Blot hybridization analysis of Aα, Aβ, Eβ, and Ii RNAs from the R8 and R8205 cells. Blots of RNA isolated from these two lines were hybridized to the indicated probes. Hybridization to the H-2 (class I) probe shows the amounts of RNA from the R8 and R8205 cell lines loaded in each lane.

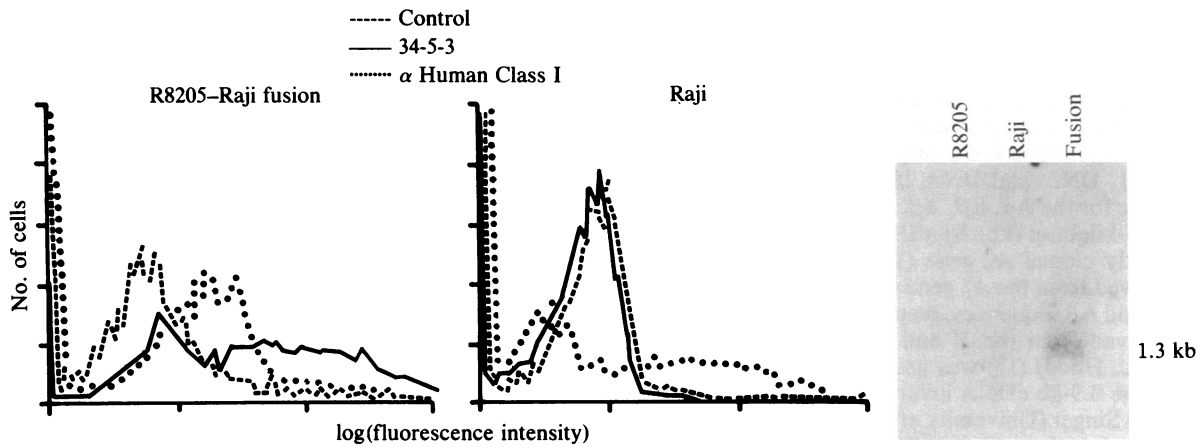

FIG. 2. Activation of murine Ia expression by somatic cell hybridization. (*Left*) Flow cytometry analysis of expression of I-$A^{b,d}$ on a somatic cell hybrid between the R8205 cell and the human B-cell line Raji; control staining of the parent Raji line is also shown. Reactivities with the anti-I-$A^{b,d}$ 34-5-3 and with an anti-(α) human class I mAb are shown. Control is the fluorescence histogram obtained with the fluorescein-conjugated goat anti-mouse Ig reagent alone. (*Right*) Blot hybridization analysis of Eβ RNAs from the R8205–Raji cell fusion (lane 3) and from the two parent cell lines (lanes 1 and 2). A mouse Eβ probe was used and the filters were washed at 60°C to avoid cross-hybridization of the mouse probe to human class II genes. Class II-specific murine Eβ RNA was detectable only in the interspecies hybridoma and not in either of the two parent cell lines.

filters were washed at 60°C to avoid cross-hybridization of the murine probe to human class II genes. The presence of the human genome resulted in the expression in the R8205 cells of the murine class II genes, and both I-A and I-E antigens were expressed.

Induction of Class II Gene Expression by BSF-1. On the basis of the recent observation that BSF-1 induces Ia expression on normal resting B cells, the R8205 cells were cultured with BSF-1 purified by high-pressure liquid chromatography as described (12). The cells were then analyzed by flow cytometry for surface expression of I-$A^{b,d}$ (Fig. 3 *Left*) and total RNA was examined by blot hybridization analysis for the presence of Ia-specific mRNA (Fig. 3 *Right*). R8205 cells cultured with BSF-1 for 72 hr expressed I-$A^{b,d}$ surface antigens and contained mRNA specific for the three Ia chain genes tested (Aα, Eβ, and Ii); untreated R8205 cells did not. The parent cell line R8 also showed an increase in surface Ia expression and class II-specific mRNA in response to BSF-1 (Fig. 3 *Right*).

Incubation of R8205 with recombinant IFN-γ (Genentech, South San Francisco, CA; 20 or 100 units/ml) for 48 or 72 hr had no effect on class II gene expression, despite the presence of IFN-γ receptors on the R8205 cells, evidenced by the fact that IFN-γ was able to increase the expression of class I genes in this cell (L.H.G., unpublished data). BSF-1 had no effect on Ia expression in the macrophage-like cell line $P388D_1$ (not shown).

Induction of Class II Gene Expression by Anti-Lyb-2 mAb. B-cell Ia expression can be increased by cross-linking B-cell receptors with anti-Ig antibodies (6) or by treatment with anti-Lyb-2 antibody (E. M. Rabin, J. J. Mond, J.O., and W.E.P., unpublished data). Since the R8205 cells are surface Ig-negative, we asked whether anti-Lyb-2 could induce Ia expression. Treatment of the R8205 cells with an antibody against the Lyb-2.2 allelic form, which is expressed by both the BALB/c and C57BL/6 strains from which the R8 is derived, resulted in expression of Ia by the R8205 cells (Fig. 4). Culture with an antibody to the alternative allelic form of Lyb-2 (anti-Lyb-2.1) had no effect on R8205 cells.

DISCUSSION

Previous work on the regulation of class II gene expression has concentrated on the role either of lymphokines, such as IFN-γ (2, 3), or of *trans*-acting regulatory factors (7–9, 21). We have studied the effect of both of these stimuli on an

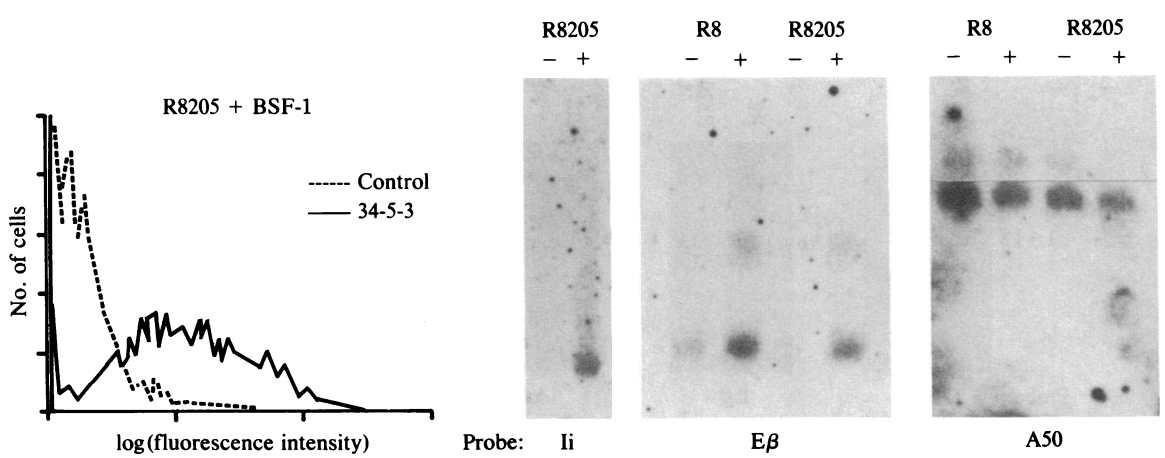

FIG. 3. Induction of class II gene expression by BSF-1. (*Left*) Flow cytometry analysis of expression of I-$A^{b,d}$ molecules on the R8205 cell after 72-hr induction with BSF-1. Reactivity with the anti-I-$A^{b,d}$ 34-5-3 mAb is shown. Control is the fluorescence histogram obtained with the fluorescein-conjugated goat anti-mouse Ig reagent alone. (*Right*) Blot hybridization of Eβ and Ii RNAs after 72-hr induction of the R8 and R8205 cells with BSF-1. Rehybridization to the A50 probe shows the amounts of RNA loaded in each lane.

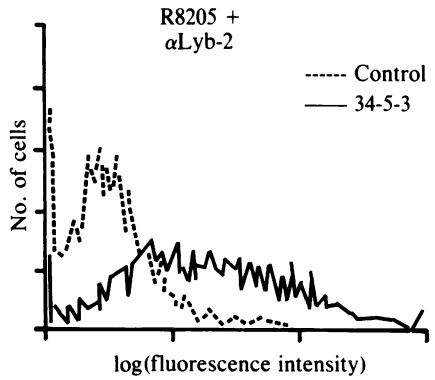

FIG. 4. Induction of class II gene expression by anti-Lyb-2 mAb. Flow cytometry analysis of the expression of I-$A^{b,d}$ after 48-hr culture of the R8205 cell with anti-Lyb-2 (Lyb-2.2, allelic form expressed by the R8 cell). Reactivity with the anti-I-$A^{b,d}$ 34-5-3 mAb is shown. Control is fluorescein-conjugated goat anti-mouse Ig alone.

Ia-negative variant of R8, an Abelson-virus-transformed pre-B-cell line. This variant cell line, R8205, contained no, or very low levels of, transcripts for all class II genes, including the nonpolymorphic *Ii* gene. Thus, this cell line differs from the Ia-negative variants described by others, which do contain mRNA specific for Ii. The K^b mutation in the R8205 cell line is probably unrelated to its lack of class II expression, since clones of the unmutagenized and nonimmunoselected parent line R8 can be isolated that are class II negative and have normal class I gene expression (S.G.G., unpublished data).

By somatic cell hybridization, we have first confirmed that an interspecies *trans*-acting regulatory factor can restore class II gene expression in the R8205 cell. In contrast to the results of Accolla *et al.* (8), both I-A and I-E antigens were expressed in our interspecies hybrids. It is possible that the apparent absence of DQ as opposed to DR antigens in their hybrids might reflect quantitative rather than qualitative differences. The interspecies fusion of the murine variant cell line with a human, rather than another murine, cell was deliberately performed to take advantage of the fact that most mouse–human hybrids are unstable and randomly lose human chromosomes at a high rate. It is indeed possible that the two hybrids negative for murine class II gene expression have lost the human chromosome that is responsible for the production of the postulated *trans*-acting factor. By correlating retention or loss of human chromosomes with the expression of the murine class II genes in the interspecies hybrids, it should be possible to map the *trans*-acting regulatory element to a single human chromosome.

BSF-1 was initially described as a required costimulant, together with low concentrations of anti-IgM antibodies, for DNA synthesis in B cells (26). Recently, BSF-1 has also been shown to increase the levels of Ia expression on resting B cells (4), increase the volume of resting B cells (27), and speed their entry into S phase (28, 29). BSF-1 has therefore been recognized to function principally as an activation and an Ia-inducing factor on resting B cells. A B-cell tumor line that is BSF-1 inducible has not heretofore been identified. We have shown here that BSF-1 induces expression of the class II genes in the Ia-negative R8205 cell and increases class II gene expression in the parental R8 cell. Induction of expression of a previously completely silent gene may not be equivalent to increased expression of an operating gene, and for this reason the parental line R8, which we have shown is BSF-1-inducible as well, may eventually prove a better model for normal B-cell differentiation than the R8205.

Ia-negative cells in which the defect in expression is related to a gene located outside the MHC [the *trans*-acting gene(s)], such as the SCID cells, have been shown to be unresponsive to IFN-γ, suggesting that inducibility by this lymphokine may require the normal functioning of the *trans*-acting regulatory gene (10). In contrast to these results, BSF-I did induce class II gene expression in the R8205 cell. One explanation for this difference is that BSF-1 may substitute for or mimic a *trans*-acting factor gene product, or it may itself activate a *trans*-acting gene. Alternatively, the defect in the R8205 cell line may be different than in other cell lines described that do express Ii and are not lymphokine inducible. Indeed, *trans* complementation has been shown between an Ia-negative T-cell line and an Ia-negative variant B cell, providing evidence for multiple regulatory factors (9).

We also determined the activity of an antibody to the surface marker Lyb-2, present on pre-B cells and on the R8205 cell, as well as on B cells. An antibody directed against the allelic form of Lyb-2 present on the R8205 cell was able to induce class II gene expression in the R8205 cells, while an antibody specific for an alternative allelic form did not affect class II gene expression. The Ia-inducing ability of both BSF-1 and Lyb-2 could be explained by the recent proposal that the Lyb-2 cell surface structure is physically related to the receptor for BSF-1 (30). Alternatively, both these signals, perturbation of the Lyb-2 cell surface antigen by the anti-Lyb-2 antibody and occupation of the BSF-1 receptor by BSF-1, could result in the release of common "second messengers" or mediators.

In agreement with those of others (7–9), our results suggest that the expression of class II genes is under the control of *trans*-acting regulatory factor(s). The concept of factors coordinately and positively regulating a set of genes has been suggested on the basis of results in other systems, such as the family of glucocorticoid-responsive genes (31). Coordinate down-regulation of a negative factor repressing mRNA production seems less likely in our system as well as that of others (8, 22). The concept of a *trans*-acting regulatory factor for all class II genes is consistent with the regulation of these same genes by BSF-1 and anti-Lyb-2, and it again suggests the possibility of a shared target sequence, possibly conserved 5′ structural elements of the class II genes, or common second messengers. Further studies using the R8 and R8205 cells will be useful in identifying the site and mechanism of the Ia-inducing action of BSF-1, as well as the structural sequences responsible for inducible expression.

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