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Immunology

Photoaffinity labeling demonstrates binding between Ia molecules and nominal antigen on antigen-presenting cells

(class II histocompatibility antigens/major histocompatibility complex/insulin/T-cell antigen receptor)

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ABSTRACT **We have used radioiodinated photoreactive bovine insulin as antigen to examine the molecular nature of immunogenic complexes that form on antigen-presenting cells. The probe was allowed to bind to either insulin-presenting B-hybridoma cells, lipopolysaccharide-stimulated blasts, or bovine insulin-specific helper-T-hybridoma cells in the dark. Samples were then exposed to light to induce crosslinkage, solubilized, and analyzed by gel electrophoresis. Two protein bands at about 36 kDa and 27 kDa were specifically labeled on antigen-presenting cells but not on helper T cells. Treatment of these bands with dithiothreitol or endo-β-*N*-acetylglucosaminidase F showed that each is composed of a single glycoprotein. These proteins are immunoprecipitable with haplotype-specific but not control anti-Ia antibodies. This identifies the labeled bands as the α and β subunits of class II major histocompatibility antigens. We conclude that a molecular complex may form between Ia and antigen on antigen-presenting cells and that formation of this complex does not require the presence of a helper-T-cell antigen receptor.**

Antigen-presenting cells (APCs) bind and present antigens to immunocompetent T lymphocytes "in the context of" Ia molecules (1, 2). However, the molecular nature of the immunogenic complexes formed on the surface of these cells is unknown. Evidence from functional assays supports the notion that antigen or fragments of processed antigen bind directly to Ia molecules to form complexes that are recognized by the helper-T (T_h)-lymphocyte antigen receptor (TcR) (2–7). Although there is some biochemical evidence for an interaction between antigen and the polymorphic Ia glycoproteins (8–10), this has been difficult to demonstrate directly (11, 12). As a result, alternative hypotheses have been developed, which suggest that foreign antigens and Ia glycoproteins exist as independent molecules on the plasma membranes of an APC and are brought together only as a consequence of binding to a TcR with specificity for both molecules (2, 13, 14). In the present study, we used the technique of photoaffinity labeling to demonstrate that Ia glycoproteins do indeed bind antigen on APCs and that this molecular complex is formed in the absence of a TcR.

MATERIALS AND METHODS

Mice. Mice were bred and maintained at the University of Toronto. Strains and their haplotypes are given in the figure legends. Splenic lymphocytes were obtained from female mice at 8–12 weeks of age.

Cell Lines. The B-cell hybridomas TA3 ($Ia^{k/d}$) (15) and LB ($Ia^{b/d}$) (16) present bovine insulin on I-A^d and I-$A^{d/b}$, respectively, to appropriately restricted, bovine-insulin-specific T-cell hybridomas, including A20.1.5.3 (A20, I-A^d) (prepared as in ref. 15) and AF3G7 (AF, I-A^b) (17). Lipopolysaccharide (LPS)-stimulated blasts were prepared by culture of splenic lymphocytes from various strains of mice for 48 hr in RPMI 1640 medium containing 2-mercaptoethanol (50 μM), penicillin (100 units/ml), streptomycin (100 μg/ml), *Escherichia coli* LPS (25 μg/ml), and 10% fetal bovine serum. Blasts were isolated from culture by density centrifugation on Ficoll-Hypaque.

Preparation of Photoreactive Insulins. Photoreactive antigen (B^{29}AZAP) was prepared by derivatization of Lys-29 of the B chain of bovine insulin with *N*-[4-(4′-azido-3′-[^{125}I]iodophenylazo)benzoyl]-3-aminopropyl-*N*′-oxysuccinimide (New England Nuclear) (18) and then purified by HPLC. Details of the preparation and purification of the insulin derivative are described elsewhere (19).

Photoaffinity Labeling. Hybridoma cells or LPS-stimulated blasts were harvested from culture, washed three times in serum-free RPMI 1640, and resuspended to 10^7 cells per ml in phosphate-buffered saline (PBS: 0.1 M phosphate/0.15 M NaCl, pH 7.4) containing aprotinin (200 μg/ml). Three hundred microliters of cell suspension was incubated with 100 μl of B^{29}AZAP (≈0.34 nM) for 5 min to 3 hr in 37°C at 5% CO_2, in the dark. The specificity of binding was determined by the addition of unlabeled native bovine insulin (final concentration 10 μg/ml) to control samples. Photoinduced crosslinking was carried out by exposing the samples to a focused light source of a 100-W high-pressure mercury lamp for 15 sec. The cells were washed three times in PBS containing soybean trypsin inhibitor (SBTI) (100 μg/ml) and bacitracin (0.8 mg/ml). Samples were solubilized by boiling for 20 min in 62.5 mM Tris·HCl, pH 6.8/3% $NaDodSO_4$/10% (vol/vol) glycerol and were analyzed by $NaDodSO_4$/PAGE in 5–10% polyacrylamide gradient gels. Autoradiographs of the gels were obtained on Kodak X-Omat AR film, using a DuPont Cronex Lightning-Plus intensifying screen during a 2-day exposure. Size markers used were myosin heavy chain (200 kDa), β-galactosidase (116 kDa), phosphorylase (93 kDa), bovine serum albumin (66 kDa), and ovalbumin (45 kDa).

Reduction and Deglycosylation of Polypeptides. The 42-kDa and 33-kDa radiolabeled bands were cut from dried autoradiographed $NaDodSO_4$/PAGE tracks prepared as described above. To analyze the proteins under reducing conditions, gel pieces were rehydrated in 2% $NaDodSO_4$ sample buffer with or without 50 mM dithiothreitol, and the proteins were electrophoresed again. For treatment with endo-β-*N*-acetylglucosaminidase F (Endo F), gel bands were rehydrated in 100 μl of 10 mM acetate/150 mM NaCl/10 mM EDTA/0.1%

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Abbreviations: APC, antigen-presenting cell; B^{29}AZAP, bovine insulin derivatized at B-chain residue Lys-29 with *N*-[4-(4′-azido-3′-[^{125}I]iodophenylazo)benzoyl]-3-aminopropyl-*N*′-oxysuccinimide; Endo F, endo-β-*N*-acetylglucosaminidase F; LPS, bacterial lipopolysaccharide; mAb, monoclonal antibody; TcR, T-cell antigen receptor; T_h cell, helper T cell.

$NaDodSO_4$/1% (vol/vol) Nonidet P-40 at pH 4 (Endo F buffer) and were alternately frozen in dry ice/acetone and thawed in a 37°C water bath three times in 10-min cycles. Five microliters of buffer or of buffer containing Endo F (5 units) was added to each gel slice, and the samples were incubated 16 hr at 37°C with shaking. The reaction was stopped by the addition of 50 μl of 2% $NaDodSO_4$ buffer and the samples were reanalyzed by $NaDodSO_4$/PAGE.

Immunoprecipitations. Cells were photoaffinity-labeled using B^{29}AZAP as described above except that solubilization was done in PBS containing 2% (vol/vol) Triton X-100 and 2 mM phenylmethylsulfonyl fluoride by incubation for 90 min at 4°C. The solubilized cell suspension was centrifuged for 30 min at 11,000 × *g*, and the supernatant was used for immunoprecipitation. Two hundred microliters of the solubilized cell supernatant was incubated at 4°C overnight with either 12 μl of serum, 6 μl of ascites, or 10 μg of purified immunoglobulin. Samples were incubated 1 hr with 10 μl of packed, glutaraldehyde-fixed *Staphylococcus aureus* cells, and the *S. aureus* cells were then washed three times in buffer (pH 7.4) containing 10 mM Tris, 150 mM NaCl, and 50 mM Hepes. Immunoprecipitated proteins were eluted by boiling for 20 min in 2% $NaDodSO_4$ sample buffer and were analyzed by $NaDodSO_4$/PAGE.

RESULTS AND DISCUSSION

Mouse and guinea pig T_h-cell responses to bovine insulin are classic examples of an *Ir* gene-controlled immune response (20, 21). In the mouse, the antigenic portion of bovine insulin appears to reside in a cluster of predominantly polar residues on the external surface of the molecule. These residues include Val-10, Ser-9, Ala-8, and Glu-4 of the A chain and Asn-3, Ala-30, and Lys-29 of the B chain. The response appears to be I-A-restricted in the $H\text{-}2^b$ and $H\text{-}2^d$ haplotypes, whereas the *H-2 s, a, q, r,* and *k* haplotypes are nonresponders to bovine insulin (15, 21, 22). For this study, a radioiodinated photoaffinity probe, B^{29}AZAP, was prepared by derivatization of Lys-29 of the B chain of bovine insulin with *N*-[4-(4′-azido-3′-[^{125}I]iodophenylazo)benzoyl]-3-aminopropyl-*N*′-oxysuccinimide (18) and then purified by HPLC. The probe was incubated with various types of APCs and/or with bovine-insulin-specific T_h hybridomas. After the cells were allowed to bind B^{29}AZAP, samples were illuminated to effect covalent crosslinking. The cells were then washed, solubilized, and analyzed by $NaDodSO_4$/PAGE. This procedure permits the identification of antigen-binding molecules by their specific crosslinking to radioactive antigen.

Two B-cell hybridoma APCs were used; the TA3 ($Ia^{k/d}$) and LB ($Ia^{b/d}$) lines present bovine insulin on I-A^d and I-$A^{d/b}$, respectively, to appropriately restricted, bovine-insulin-specific T-cell hybridomas, including A20 (I-A^d) and AF (I-A^b). We have previously shown (23) that both of these T-cell hybridomas recognize an insulin photoprobe prepared by derivatization of bovine insulin at B-chain residues Lys-29 or Phe-1 with a nonradioactive photoreactive activated ester, *p*-azidobenzoyl-*N*-hydroxysuccinimide. Their response to the photoprobes was equivalent to that produced by native bovine insulin. This demonstrates that such insulin photoprobes are functionally active and can be used to examine their interactions with Ia molecules on APCs.

Two major protein bands were photoaffinity-labeled by B^{29}AZAP on TA3 (Fig. 1, lanes A and B; Fig. 4, lane S) and LB (Fig. 4, lane T) cells. The specificity of labeling was demonstrated by competitive inhibition with excess unlabeled native bovine insulin at 10 μg/ml (Fig. 1, lane B). The average molecular masses of the two labeled proteins, estimated from nine $NaDodSO_4$/polyacrylamide gradient gels run under nonreducing conditions, were 42 kDa and 33 kDa. The apparent molecular mass of these proteins includes the mass of insulin (5734 Da) or some processed portion of the antigen. Thus, the molecular mass of the antigen-binding proteins should be adjusted to 36.3–42 kDa and 27.3–33 kDa, respectively. Labeling of these bands could be detected after 10 min of incubation of the cells with B^{29}AZAP, and the intensity of labeling increased steadily over 4 hr. These bands were not seen when A20 or AF T cells (Fig. 1, lanes C–F) were photoaffinity labeled; other bands in the 45- to 60-kDa region, attributable to proteins derived from T_h cells, may be seen in these samples. The specificity of labeling of these latter bands is not known. They are included here to show that the 42-kDa and 33-kDa B-cell proteins are not found on T_h cells.

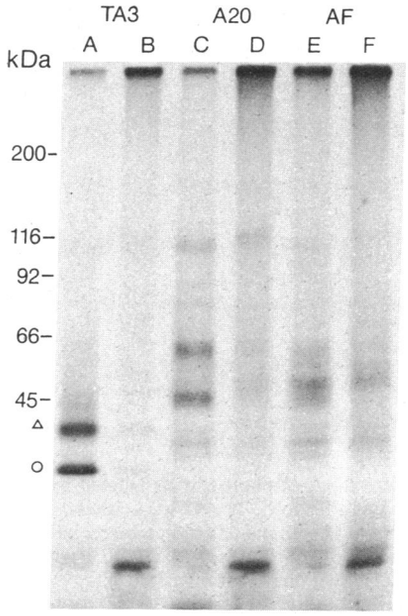

FIG. 1. Photoaffinity labeling of B- and T-cell hybridomas. TA3 (lanes A and B), A20 (lanes C and D), and AF (lanes E and F) hybridoma cells were incubated with B^{29}AZAP for 3 hr at 37°C. The specificity of binding was determined by the addition of unlabeled native bovine insulin (final concentration 10 μg/ml) to control samples (lanes B, D, and F). Samples were photolyzed, solubilized, and analyzed by $NaDodSO_4$/PAGE. △, 42-kDa band; ○, 33-kDa band.

The conditions of labeling used in this study are not optimal for the demonstration of insulin receptors. When binding was allowed to occur overnight at 4°C, the insulin receptor could be easily detected on the AF and A20 T-cell lines and on splenic B-cell blasts stimulated by LPS for 48 hr. The cell lines TA3 and LB are not rich in insulin receptors, although faint bands corresponding to insulin-receptor proteins were sometimes seen.

After $NaDodSO_4$/PAGE under nonreducing conditions, the 42-kDa and 33-kDa bands were cut from slab gels, incubated for 30 min in $NaDodSO_4$ sample buffer with or without dithiothreitol, and electrophoresed again in a 5–10% gradient gel. Reduction caused only a minor shift in the apparent size of the two proteins (Fig. 2, lanes A–D). This shift is most likely due to the loss of the A chain of insulin. Thus, we conclude that the two photoaffinity-labeled bands represent single polypeptide chains. When the 42-kDa and 33-kDa bands were subjected to deglycosylation by incubation at 37°C for 16 hr with Endo F (24) at pH 4 (Fig. 2, lanes E–G) or at pH 9 (data not shown), both bands showed increased electrophoretic mobility. Thus, both represent glycoproteins. Two bands were apparent after the 42-kDa band was deglycosylated. This could indicate either incom-

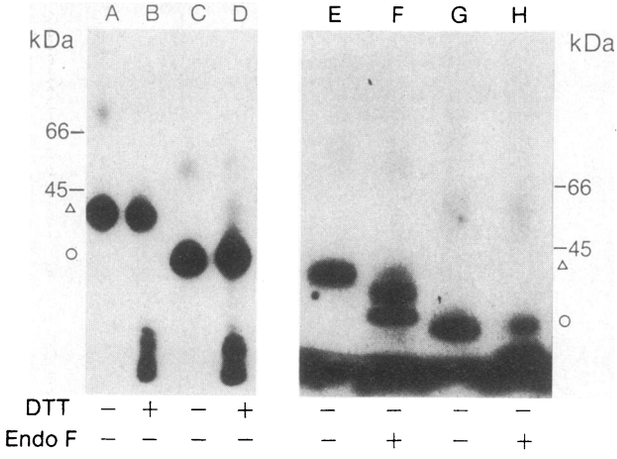

FIG. 2. Treatment of photoaffinity-labeled proteins with dithiothreitol (DTT) and Endo F. The 42-kDa (lanes A, B, E, and F) and 33-kDa (lanes C, D, G, and H) radiolabeled bands were cut from dried, autoradiographed $NaDodSO_4$/PAGE tracks prepared as for Fig. 1. Gel pieces were rehydrated and treated either in $NaDodSO_4$ sample buffer (lanes A and C), in $NaDodSO_4$ sample buffer containing 50 mM DTT (lanes B and D), Endo F buffer (lanes E and G), or Endo F buffer containing Endo F (lanes F and H) and were electrophoresed as described for Fig. 1. △, 42-kDa band; ○, 33-kDa band.

plete deglycosylation of the glycoprotein or the presence of two differentially glycosylated molecules in the original band.

Because of the similarity in size and endoglycosidase sensitivity between the two insulin-binding glycoproteins and the α and β chains of the I-A and I-E class II histocompatibility antigens (25), samples of B^{29}AZAP-labeled TA3 and LB cells were immunoprecipitated with I-A- or I-E-specific antibodies, using fixed *S. aureus* cells. Both glycoproteins were immunoprecipitated from TA3 cell lysates by a polyclonal anti-Ia alloantiserum (A.TH anti-A.TL) specific for $I\text{-}A^{k,b,d}$ and $I\text{-}E^{k,d}$ (26) (Fig. 3, lane B) but not by the inverse serum (A.TL anti-A.TH) specific for $I\text{-}A^{s}$ (26) (lane C) or normal mouse serum (lane A). Identical results were obtained using LB cells (data not shown). These results clearly show that the photoaffinity-labeled glycoproteins are I-A and/or I-E molecules. Based on the radioactivity of the bands cut from the $NaDodSO_4$ slab gels and on the specific activity of the B^{29}AZAP (2200 Ci/mmol; 1 Ci = 37 GBq), we estimate that 0.1–1 Ia molecule is crosslinked per cell. Since the

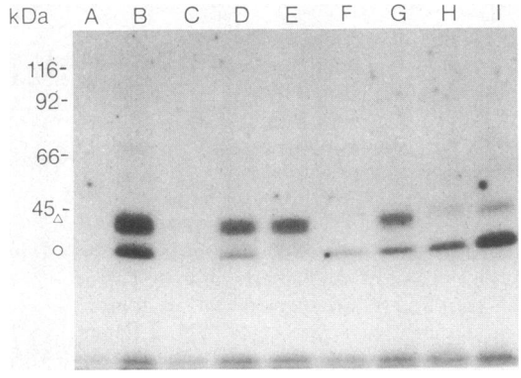

FIG. 3. Immunoprecipitation of photoaffinity-labeled Ia molecules. TA3 cells were photoaffinity-labeled using B^{29}AZAP and were immunoprecipitated using normal mouse serum (lane A), A.TH anti-A.TL serum (lane B), A.TL anti-A.TH serum (lane C), JF01-6 serum (lane G), 11.5 ascites (lane F), 10.3 ascites (lane H), MK.D6 ascites (lane I), or purified mAbs 40B (lane D) and 40D (lane E). Samples were analyzed by $NaDodSO_4$/PAGE. △, Ia α chain; ○, Ia β chain.

efficiency of photocrosslinking is 10–15%, this estimate could suggest that 1–10 molecules of antigen are bound to Ia per cell.

Immunoprecipitations were also performed with several additional antisera and monoclonal antibodies (mAbs) selected for different reactivities with either α or β subunits of I-A and I-E molecules. Each of these mAbs gave a unique pattern of precipitation from the same preparation of B^{29}AZAP-labeled TA3 cells. Approximately equal amounts of photoaffinity-labeled Ia α and β chains were precipitated by the polyclonal A.TH anti-A.TL serum, the α-chain band being composed of a doublet. Similar results were obtained using a $(B10.D2 \times C3H.Q)F_1$ anti-B10.AQR alloantiserum (JF01-6) reported (28) to be $I\text{-}A^{k}_{\beta}$-specific with potential but undetected reactivity with $I\text{-}A^{k}$ α chains (Fig. 3, lane G). The 40B (anti-$I\text{-}E^{k,d}_{\beta}$) and 40D (anti-$I\text{-}E^{k,d}_{\alpha}$) mAbs (27) precipitated a prominent α-chain doublet and a less apparent (absent in some experiments) β-chain band (Fig. 3, lanes D and E). In immunoprecipitates of the 11.5 and 10.3 (anti-$I\text{-}A^{k}_{\beta}$) mAbs (28) (lanes F and H) and the anti-$I\text{-}A^{d}_{\beta}$ mAb MK-D6 (28) (lane I), the β-chain band was predominant.

Two factors may contribute to the disparate results obtained with these mAbs. First, if each Ia molecule binds only one molecule of antigen, then when a given Ia molecule is photoaffinity-labeled with antigen, the probe has the potential to crosslink either the α or the β chain of that Ia molecule but not both. If the site of crosslinking is close to or in the Ia epitope recognized by a particular mAb, then one would expect the binding of that mAb to be inhibited by the bound antigen. Thus, the mAb would be expected to precipitate only Ia molecules crosslinked on the alternate chain or at a site on the same chain but not including the mAb binding site. This effect would not be seen in the case either of a polyclonal antibody or of a mAb that binds to an epitope distinct from any antigen-crosslinking site. The second factor could be the differential labeling of the I-A and I-E antigens in the k and d haplotypes; i.e., the result is consistent with the interpretation that the $I\text{-}A^{k}$ molecule is poorly labeled and only labeled on the β chain, that $I\text{-}E^{k}$ and $I\text{-}E^{d}$ are labeled primarily on the α chain, and that $I\text{-}A^{d}$ is labeled primarily on the β chain.

LPS-stimulated blasts were prepared from 16 strains of inbred mice with different parental and recombinant Ia haplotypes. LPS has been shown (29) to enhance membrane-associated Ia expression of splenic B cells. The blast cells were photoaffinity-labeled with B^{29}AZAP, washed, solubilized, and analyzed by $NaDodSO_4$/PAGE without immunoprecipitation. Labeling of Ia molecules derived from all of the mouse strains tested was observed (Fig. 4). However, the degree of labeling, particularly of the α chain, differed among strains (both in the intensity of the bands and in whether the α chain appeared as a doublet or as a single band). For example, the BALB/c (Fig. 4, lane C), B10.HTT (lane Q), and B10.A (lane J) LPS blasts showed increased intensity of labeling of the α-chain bands, and the bands themselves were less discrete. We are cautious in the interpretation of this result, since LPS stimulation may cause different degrees of Ia expression among different mouse strains, and there may be some variations in cell number or sensitivity to manipulation during the experimental procedure. Nonetheless, when four strains that showed the most disparate insulin-Ia binding patterns were tested in a second experiment, the result was identical to that shown. Since mouse Ia β chains are more polymorphic than the Ia α chains, it has been proposed that the β chain is a more likely candidate for the antigen-binding subunit of the presentation complex (reviewed in ref. 25). Our B^{29}AZAP probe has a molecular size and flexibility that are expected to allow labeling of adjacent subunits of a molecular complex even if only one of those subunits represents the primary binding site of the ligand. For example, it has been

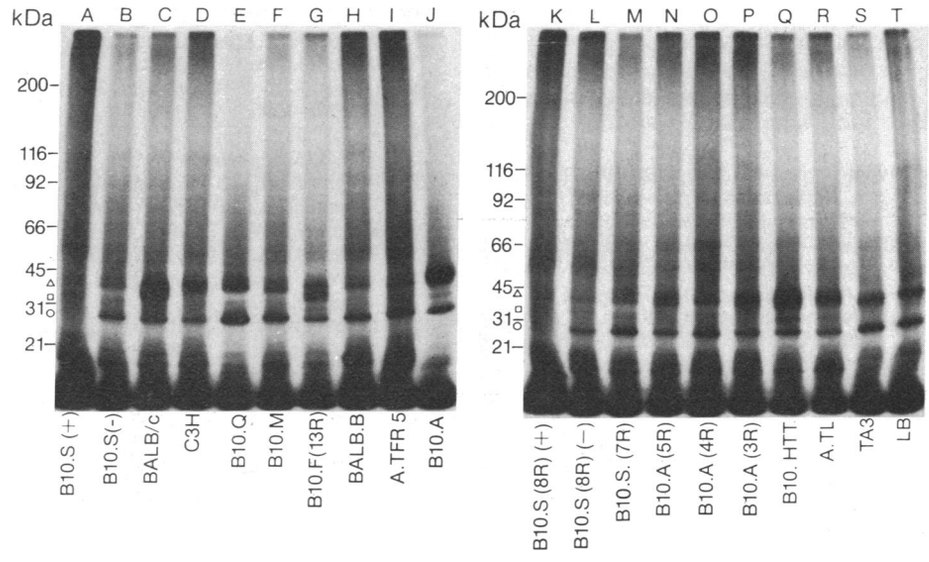

FIG. 4. Photoaffinity labeling of LPS-stimulated blast cells. TA3 and LB cells and LPS-stimulated blast cells from the indicated mouse strains were photoaffinity-labeled with B^{29}AZAP and analyzed by $NaDodSO_4$/PAGE. Lanes: A and B, B10.S (I-A^s, I-E^-); C, BALB/c (Ia^d); D, C3H (Ia^k); E, B10.Q (Ia^q); F, B10.M (Ia^m); G, B10.F(13R) (Ia^p); H, BALB.B (I-A^b, I-E^-); I, A.TFR5, (I-A^f, I-$E^{f/k}$); J, B10.A (Ia^k); K and L, B10.S(8R) (I-A^k, I-E^-); M, B10.S(7R) (I-A^s, I-E^-); N, B10.A(5R) (I-A^b, I-E^k); O, B10.A(4R) (I-A^k, I-E^b); P, B10.A(3R) (I-A^b, I-E^k); Q, B10.HTT (I-A^s, I-E^k); R, A.TL (I-A^s, I-E^k); S, TA3 ($Ia^{k/d}$); T, LB ($Ia^{b/d}$). An unlabeled native insulin control was prepared for two strains of mice (+, lanes A and K). △, Ia α chain; ○, Ia β chain; □, 35-kDa protein.

shown (19) that this probe will label both the 130-kDa and the 90-kDa subunits of the insulin receptor, although the 130-kDa subunit is the insulin-binding subunit. In the current experiments, the degree of β-chain labeling obtained appears to be quite consistent, in contrast to α-chain labeling, which shows more variability between strains. This suggests that the orientation of the antigen differs when the antigen is bound to different polymorphic Ia molecules, in some cases allowing contact between the insulin B-chain residue Lys-29 and the Ia α chain, while in other cases the probe may be in a less optimal position for α-chain crosslinking. Four of the strains shown in this experiment, BALB.B (Fig. 4, lane H), B10.S (lanes A and B), B10.S(7R) (lane M), and B10.S(8R) (lanes K and L), do not express an I-E α chain and therefore do not have surface I-E molecules (30, 31). Thus the Ia bands seen with these strains are I-A molecules. Since B10.S(8R) and B10.A mice are identical at I-A and differ in the expression of I-E α chains (B10.A, I-E_α^+; B10.S(8R), I-E_α^-), the relative difference in α-chain labeling between these two strains is most likely due to the contribution of the I-E molecule in B10.A.

Two mechanisms have been proposed to explain the manner in which Ia molecules restrict the ability of T_h cells to respond to foreign antigen. One hypothesis suggests that restriction is caused by the inability of a particular antigenic fragment to form an appropriate complex with Ia molecules on the nonresponder APC (reviewed in ref. 7). Alternatively, it has been proposed that nonresponder strain animals lack T_h cells that are specific for the complex formed between the antigen and the nonresponder Ia (32, 33). It is of interest that no marked difference in labeling was seen among the I-A^+, I-E^- strains, only one of which (BALB.B) is a high responder to bovine insulin. It is not possible, however, to interpret these results in support of one or the other hypothesis of major histocompatibility complex restriction, for the following reasons. First, the photoreactive antigen is somewhat more hydrophobic than native bovine insulin and its lysine ϵ-amino group is derivatized. It is possible that this difference is sufficient to alter the restriction pattern of the antigen. There is evidence that hydrophobic regions of the antigen are important in antigen–Ia interaction (reviewed in ref. 34). We will be able to resolve this question only by immunization of nonresponder strains with B^{29}AZAP. Second, we do not know to what degree the insulin molecule has been processed by the APC before it binds and is photocrosslinked to Ia molecules. It has been reported that the insulin molecule does not require processing for presentation (35), and the molecular masses of our photoaffinity-labeled proteins suggest that they are crosslinked to an antigen that approximates native insulin in size. We cannot exclude the possibility, however, that the Ia–antigen complex labeled in these experiments does not represent the fragment of insulin for which T_h–APC major histocompatibility complex restriction is seen. It has been proposed that the determinants recognized by T_h cells differ from those recognized by suppressor T (T_s) cells (36). The binding of insulin to Ia antigens of both high- and low-responder strains may not be surprising in view of the observation that insulin-responsive T_s cells have been found in some nonresponder haplotypes (37). The use of photoreactive probes prepared from antigenic fragments of insulin may help resolve these questions. This approach to the study of immunogenic peptide–Ia interactions differs from the equilibrium-dialysis experiments described by Babbit *et al.* (38), which show that a hen egg lysozyme hexadecapeptide binds differentially to detergent-solubilized responder and nonresponder Ia molecules. Our insulin photoprobe was labeled with ^{125}I during synthesis of the AZAP ester and has a comparatively high specific activity (2200 Ci/mmol). Moreover, photoaffinity labeling assays for nearest-neighbor interactions between proteins and not their relative affinities, which are detected by equilibrium dialysis. Third, it is possible that as a result of photocrosslinking, insulin (or insulin fragments) binds to highly polymorphic α1 and/or β1 domains of Ia α and β chains in responder haplotypes and (with perhaps lower affinity) to the less polymorphic α2 and/or β2 domains of Ia polypeptides in nonresponder haplotypes. This possibility awaits the identification of the Ia α- and β-chain amino acid residues involved in the binding of insulin.

In addition to the Ia α and β chains, another band, at about 35 kDa, was seen in some LPS-blast preparations (Fig. 4, lanes J and Q). It is possible that this band represents the I_i invariant chain that associates with Ia molecules (39).

In summary, we conclude that the Ia glycoproteins do, indeed, bind insulin as antigen. A TcR is not required to generate a primary association between Ia and nominal antigen on the APC, although stabilization of the complex by a TcR may be required for T-lymphocyte activation.

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