Proc. Natl. Acad. Sci. USA
Vol. 78, No. 7, pp. 4525–4529, July 1981
Immunology

Structural analysis of a new B-cell-differentiation antigen associated with products of the *I-A* subregion of the *H-2* complex

(X-linked B-cell defect/CBA/N mutant mouse)

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Communicated by Jack Leonard Strominger, March 23, 1981

ABSTRACT **Ia.W39 is a private specificity of the $I\text{-}A^b$ subregion of the *H-2* complex. It is selectively expressed on a subset of B lymphocytes that is absent in newborn normal and adult mutant mice carrying the *xid* gene. Immunoprecipitation and one-dimensional $NaDodSO_4$/polyacrylamide gel electrophoresis showed that the molecule bearing Ia.W39 consists of two noncovalently linked glycoproteins of apparent M_r 33,000 and 28,000. Anti-Ia.W39 serum did not preclear the Ia^b molecule; however, the conventional allo-anti-$I\text{-}A^b$ serum cleared Ia.W39 completely. In view of the identical two-dimensional gel pattern generated by the Ia.W39 and the conventional Ia^b immunoprecipitates, we believe that all Ia molecules bear the conventional specificities and only a subset would in addition express Ia.W39. Ia.W39 is probably not a carbohydrate antigen, because the antibiotic tunicamycin had no influence on its expression. It may be a conformational determinant on the A_α and A_β complex induced by the association of an unknown molecule with these chains.**

Since the discovery that immune response (Ir) genes map within the major histocompatibility complex (1, 2), the *I* region has been a focus of attention for immunologists. Ia specificities have been associated with the *I* region of the histocompatibility complex in many mammalian species (3–10). The functional role of the Ia antigens is still not fully understood. Many speculations have been made about their role as cell interaction molecules (11–13) or as products of the *Ir* genes themselves (14). The Ia molecule, in every species studied so far, is composed of two noncovalently linked glycoproteins that are products of loci in the *I* region, namely, an α chain of $M_r \approx 33{,}000$ and a β chain of $M_r \approx 28{,}000$ (for review, see ref. 15). A third polypeptide, the Ia-associated invariant chain (Ii) is found in murine (16) and human (17) Ia immunoprecipitates. In the mouse, Ia antigens are expressed on more than 95% of B cells (4), 30–50% of macrophages (18, 19) and dendritic cells (20), and on a low, serologically and biochemically undetectable number of helper (21) and suppressor (7) T cells. Recently, a new Ia specificity, Ia.W39, has been defined that is private for $I\text{-}A^b$ and has a unique tissue distribution pattern (22). It is a B-cell-differentiation antigen whose expression is controlled by a gene on the X chromosome (*xid*) (23) like other B-cell-differentiation markers—e.g., Lyb3 (24), Lyb5 (25), and Lyb7 (26). It is expressed on $\approx$50% of mature splenic B cells, which are absent in mice carrying the defective *xid* gene. It is also expressed on a subset of macrophages, and it is essential for optimal presentation of beef insulin, an antigen that is under Ir control mapping in the $I\text{-}A^b$ region (27), to immune T cells. In this paper, we compare the structure of Ia.W39 with that of the conventional Ia molecule. The results suggest that at least two kinds of Ia molecules are present that contain A_α and A_β chains.

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MATERIALS AND METHODS

Mice. C57BL/6 (B6) mice, 8–10 weeks old, were purchased from The Jackson Laboratory. CBA/N mice and F_1 hybrid progeny of crosses between CBA/N and B6 mice were bred in the animal facilities of Tufts University School of Medicine from breeding stock originally obtained from the National Institutes of Health. The B10.A(5R) and B10.A(4R) mice were a gift from Martin Dorf, Harvard Medical School.

Antisera. $(AxB10.A)F_1$ anti-B10.A(5R) antiserum was obtained from the Research Resource Branch, National Institute of Allergy and Infectious Diseases, and its anti-K^b activity was absorbed with EL-4 tumor cells. $(LP \times A.TH)F_1$ anti-A.TL (anti-$I\text{-}A^k$) was a gift of Martin Dorf, Harvard Medical School. Anti-Ia.W39 was prepared by immunizing $(CBA/N \times B6)F_1$ male mice with B6 spleen cells as described (22).

Microcytotoxicity Assay. Cell suspensions were treated with low ionic strength buffer (28) to lyse the dead cells, which resulted in 95–98% live cells. Cells at $5 \times 10^5/10\ \mu l$ were incubated for 20 min on ice with 25 μl of antiserum or normal mouse serum (NMS) at various dilutions in V-bottom microtiter plates (Linbro, Titertek). Then, 35 μl of preabsorbed rabbit C at a dilution of 1:6 (final dilution 1:12) was added, and the plate was incubated at 37°C on a rocker for 0.5 hr. The C reaction was stopped by addition of 80 μl of cold medium, and the viability counts were performed with phase-contrast microscopy. The percent specific killing was calculated as (% dead cells with antiserum − % dead cells with NMS) × 100/(100 − % dead cells with NMS).

Metabolic Labeling Procedure with $[^{35}S]$Methionine. Cells (2×10^7) were pelleted and suspended in 15 ml of methionine-free RPMI 1640/10% dialyzed fetal calf serum/0.5 mCi of $[^{35}S]$methionine (800 Ci/mmol, 1 Ci = 3.7×10^{10} becquerels; New England Nuclear). The cells were incubated at 37°C in 5% CO_2/95% air for 5–24 hr, pelleted, and lysed by spinning in a Vortex and addition of 2 ml of 0.5% Triton X-100/1 mM α-toluenesulphonyl fluoride/0.25 mM dithiothreitol/10 mM Tris base, pH 7.4. The mixture was incubated at 4°C for 0.5 hr and then centrifuged at 50,000 rpm (g average = 150,000). The resulting supernatant was used for the immunoprecipitation studies.

Tunicamycin Treatment. Cells $(5 \times 10^7/ml)$ were suspended in methionine-free RPMI 1640/10% dialyzed fetal calf serum and incubated for 1 hr at 37°C with tunicamycin (2–10 μg/ml) (a gift of Hide Ploegh, Harvard University). This antibiotic specifically blocks N-linked glycosylation of asparagine residues of glycoproteins by inhibiting the synthesis of the lipid-linked precursor oligosaccharide (29, 30) but has little effect on protein synthesis (incorporation of $[^{35}S]$methionine after treatment with

Abbreviations: Ir, immune response; Ii, Ia-associated invariant chain; B6, C57BL/6; NMS, normal mouse serum.

tunicamycin was within the range seen in control cultures—i.e., 10–15%). The cells were then labeled with [^{35}S]methionine as described above.

Lectin Affinity Chromatography. Sepharose-coupled *Lens culinaris* agglutinin (lentil) was obtained from Vector Laboratories (Burlingame, CA). Samples of the radiolabeled cell lysates were loaded onto the lentil affinity column (0.2 ml) at the maximum flow rate. The column was preequilibrated with 0.2% Triton X-100/0.1 mM $MgCl_2$/0.14 M NaCl/0.25 mM dithiothreitol/10 mM Tris base, pH 7.8 (lectin buffer) and washed with 5–10 column volumes of lectin buffer after application of the sample. α-Methyl mannoside (Sigma) at 3% (wt/vol) in 1 ml of lectin buffer was run into the column, which was then turned off and allowed to stand for 30 min. The specifically bound material was eluted by using the same sugar solution. All procedures were performed at 4°C.

Immunoprecipitation with Formalin-Fixed *Staphylococcus aureus* and $NaDodSO_4$/Polyacrylamide Gel Electrophoresis. These procedures were exactly as described by Kessler (31). Samples were run on 10% $NaDodSO_4$/polyacrylamide gels, except for the tunicamycin experiment, where a discontinuous gel was used as described by Laemmli (32). This gel contained a 7–15% linear acrylamide gradient.

Two-Dimensional Polyacrylamide Gel Electrophoresis. Samples were prepared and subjected to electrophoresis according to published procedures (33). Briefly, immunoprecipitated proteins were separated on nonequilibrium pH gradient gels in the first dimension and then according to size on 10% $NaDodSO_4$/polyacrylamide gels in the second dimension.

RESULTS

Definition of the Polypeptides Recognized by Anti-Ia.W39 Serum. To test whether Ia.W39 is a classical Ia specificity, we compared its form on $NaDodSO_4$/polyacrylamide gel electrophoresis with that of the $I\text{-}A^b$ molecule. Spleen cells from the H-2^b recombinant mice B10.A(5R) ($I\text{-}A^b$) and B10.A(4R) ($I\text{-}A^k$) were labeled with [^{35}S]methionine for 24 hr and extracted with Triton X-100. The lysates were then passed over a lentil lectin column precleared with NMS and treated with formalin-fixed *Staphylococcus*. Aliquots were subjected to immunoprecipitation by using NMS, anti-Ia.W39, and anti-$I\text{-}A^b$ [B10.A(5R)] or anti-$I\text{-}A^k$ [B10.A(4R)], respectively. As shown in Fig. 1, anti-Ia.W39 serum immunoprecipitates two polypeptides specifically from the B10.A(5R) lysate. These polypeptides migrate with the gene products precipitated with conventional anti-$I\text{-}A^b$ sera. They are not precipitated from lysates of spleen cells that have an $I\text{-}A^k$ background, which confirms the specificity of Ia.W39 serum. The intensity of the immunoprecipitate obtained with anti-Ia.W39 serum is weaker than that obtained with conventional anti-Ia serum. Saturation experiments confirmed that Ia.W39 reached a plateau earlier (i.e., lower counts) than conventional Ia (results not shown). Thus, there are fewer Ia.W39 than classical Ia molecules.

In addition, a polypeptide of M_r 46,000 was present in the immunoprecipitation with anti-Ia.W39 serum. This polypeptide was found both in B10.A(5R) and B10.A(4R) spleen cells, indicating that is was not of the correct specificity. This band was detected throughout our experiments. Although the immunoprecipitations were performed on glycoproteins purified by lentil lectin affinity chromatography the molecular weight of the nonspecific band suggests that anti-actin antibodies are contained in anti-Ia.W39 serum.

Ontogeny of Expression of Ia.W39. It has been shown by serological analyses (immunofluorescent staining) that the conventional Ia molecule is expressed during the first few days of life (34). We confirmed this by cytotoxicity studies on spleen cells from B6 mice at various ages. In contrast, we found that Ia.W39 could be detected by cytotoxicity on a significant number of spleen cells in 3-week-old mice only (Fig. 2A).

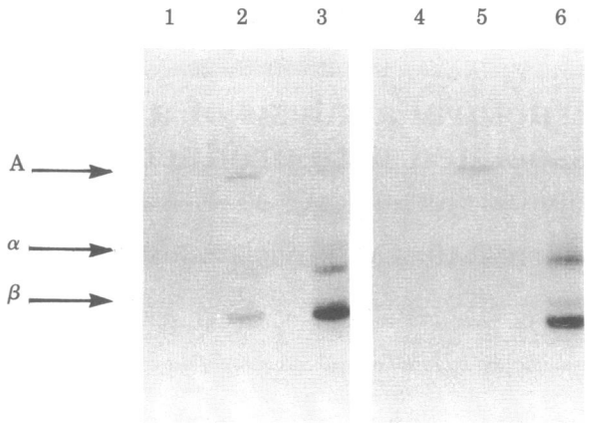

FIG. 1. Autoradiograph of $NaDodSO_4$/polyacrylamide gel electrophoresis showing expression of Ia.W39-and conventional Ia-associated polypeptides in H-2^b recombinant mice B10.A(5R) ($K^bA^bB^bJ^kE^k\text{-}C^dS^dG^dD^d$) (lanes 1–3) and B10.A(4R) ($K^kA^kB^bJ^bE^bC^bS^bG^bD^b$) (lanes 4–6). The spleen cells were metabolically labeled with [^{35}S]methionine for 24 hr. Lanes: 1 and 4, control immunoprecipitates using NMS; 2 and 5, immunoprecipitates using anti-Ia.W39 serum; 3, immunoprecipitate using anti-$I\text{-}A^b$ serum; 6, immunoprecipitate using anti-$I\text{-}A^k$ serum. Indicated bands: A, actin (M_r 46,000); α, Ia heavy chain (M_r 33,000); β, Ia light chain (M_r 28,000).

In parallel experiments, we carried out immunoprecipitations on B6 spleen cells during ontogeny. The conventional Ia molecule, immunoprecipitated from the various spleen cell lysates after 24 hr of metabolic labeling with [^{35}S]methionine and the time course of the appearance of the molecule carrying the Ia.W39 specificity are shown in Fig. 2 *B* and *C*. Although the conventional Ia molecule is readily found on neonatal spleen cells, Ia.W39 is detectable only at 8 days of age. The intensity of the precipitate spot increases for both molecules from the time of first appearance.

Is Ia.W39 Expressed on a Separate Molecule from Classical $I\text{-}A^b$? The experiments presented above on the ontogeny of Ia.W39 suggest that this specificity may be present on a population of molecules separate from classical I-A^b. We have performed a number of experiments designed to test this possibility, all of which supported the idea that there are two kinds of Ia molecules containing A_α and A_β.

Comparison of biosynthetic rates. B6 spleen cells were metabolically labeled with [^{35}S]methionine for 5 or 24 hr and processed as described above. Immunoprecipitation was then performed with conventional anti-$I\text{-}A^b$ serum, or with anti-Ia.W39 serum, or with NMS. As observed before, both anti-Ia.W39 serum and anti-$I\text{-}A^b$ serum precipitated the α and β polypeptides of M_r 33,000 and 28,000 from spleen cells labeled for 24 hr (Fig. 3, lanes 1 and 2). However, when the same experiment was performed on cells labeled for 5 hr, anti-Ia.W39 serum precipitated β chain only, although anti-$I\text{-}A^b$ serum could still precipitate both α and β chains (Fig. 3, lanes 3 and 4). This experiment suggests that the Ia.W39 α chain is synthesized at a slower rate than the α chain of the conventional Ia molecule.

Is Ia.W39 a sugar specificity? One formal possibility for the difference between Ia.W39 and classical $I\text{-}A^b$ is that the former is on a sugar molecule added to a subgroup of Ia molecules during ontogeny. To test this idea, we used the drug tunicamycin, which inhibits N-linked glycosylation. Thus, B6 spleen cells were incubated with various doses of tunicamycin for 1 hr before a 5-hr incubation with [^{35}S]methionine. Both conventional anti-

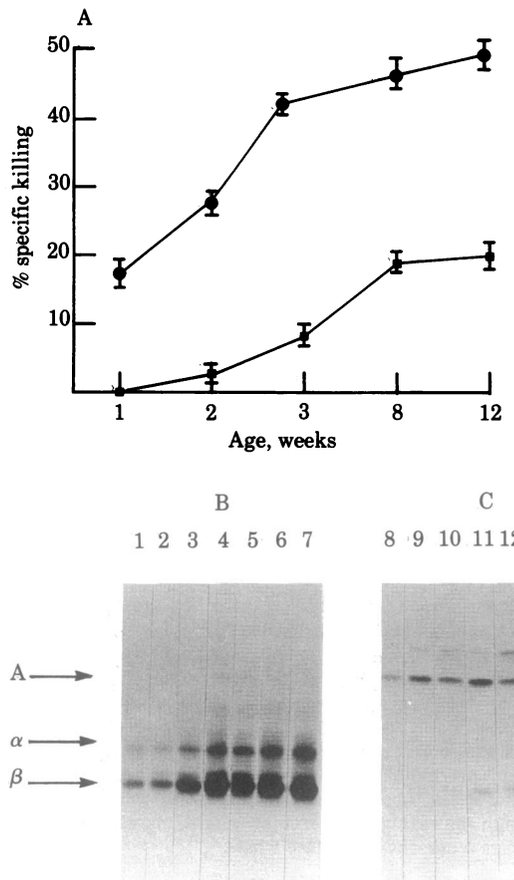

FIG. 2. Studies of the expression of Ia.W39 and conventional Ia during ontogeny of B6 mice. (*A*) Microcytotoxicity assay using anti-$I\text{-}A^b$ (●) and anti-Ia.W39 (■) sera. (*B* and *C*) Autoradiographs of $NaDodSO_4$/polyacrylamide gel electrophoresis using anti-$I\text{-}A^b$ (lanes 1–7) and anti-Ia.W39 (lanes 8–14) sera. The spleen cells were labeled with [^{35}S]methionine for 24 hr. Mice of the various age groups were pooled. Lanes: 1 and 8, neonates (<24 hr); 2 and 9, day 5; 3 and 10, day 8; 4 and 11, day 9; 5 and 12, day 11; 6 and 13, day 13; 7 and 14, day 19. Indicated bands are as described in the legend to Fig. 1.

$I\text{-}A^b$ and anti-Ia.W39 sera still immunoprecipitate proteins after treatment with this antibiotic (Fig. 4). Therefore, we concluded that the Ia.W39 specificity is not due to an unusual glycosylation in ontogeny. The same shift in apparent molecular weight is seen in both Ia^b and Ia.W39 molecules after treatment with tunicamycin, consistent with previous reports on HLA-DR molecules (35, 36). The nonspecific band, M_r 46,000, precipitated by anti-Ia.W39 serum, is insensitive to tunicamycin treatment.

Sequential immunoprecipitations. From comparison of the time of appearance during ontogeny and the rate of synthesis, we concluded that the expression of Ia.W39 is controlled by a different mechanism than that of conventional Ia specificities. To test whether Ia.W39 is expressed on a separate molecule from the other Ia specificities coded for within the $I\text{-}A^b$ region, we performed sequential immunoprecipitation experiments. B6 spleen cells were labeled for 24 hr with [^{35}S]methionine and processed as described above. As shown in Fig. 5*A*, immunoprecipitation with anti-Ia^b cleared Ia.W39, although it left a trace of Ia^b behind. On the other hand, anti-Ia.W39 serum cleared Ia.W39 and seemed to leave Ia^b intact (Fig. 5*B*). Fig. 5*C* shows the control experiment. There are three possible explanations for these findings: (*i*) Conventional anti-Ia^b serum contains activity against Ia.W39 and would, therefore, clear Ia.W39, even if this specificity is present on a separate molecule

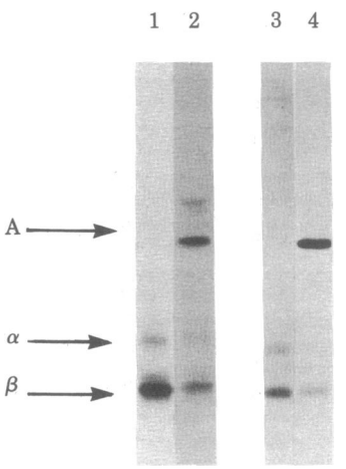

FIG. 3. Autoradiographs of $NaDodSO_4$/polyacrylamide gel electrophoresis comparing the turnover rate of Ia.W39- and conventional Ia-associated polypeptides. B6-spleen cells were metabolically labeled with [^{35}S]methionine for 24 hr (lanes 1 and 2) or 5 hr (lanes 3 and 4). Lanes: 1 and 3, immunoprecipitates using anti-$I\text{-}A^b$ serum; 2 and 4, immunoprecipitates using anti-Ia.W39 serum. Indicated bands are as described in the legend to Fig. 1.

from that expressing the conventional Ia specificities; (*ii*) there are two types of Ia molecules, one containing only the conventional specificities and the other expressing, in addition, the Ia.W39 specificity; and (*iii*) one chain—e.g., the β chain—is common to conventional Ia and Ia.W39, and the other (the α chain) is different.

Two-dimensional polyacrylamide gel electrophoresis. To clarify the results described above, we performed two-dimensional gel electrophoresis on the glycoproteins specifically immunoprecipitated from B6 spleen cells with anti-Ia.W39 serum or with an antiserum directed against $H\text{-}2K^b$, $I\text{-}A^b$, and $I\text{-}B^b$ or on NMS control precipitates (Fig. 6). Surprisingly, both the α

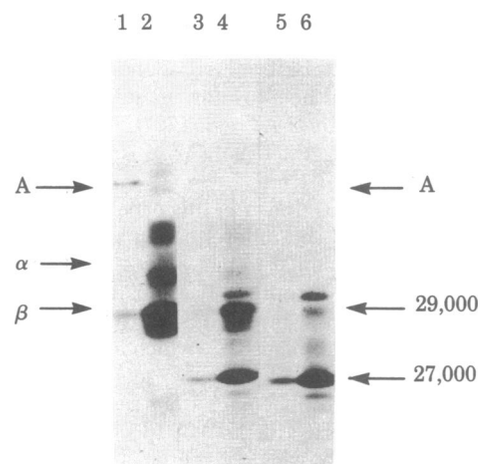

FIG. 4. Autoradiographs of $NaDodSO_4$/polyacrylamide gel electrophoresis of Ia.W39- and conventional Ia-associated polypeptides from normal or tunicamycin-treated B6 spleen cells. The cells were metabolically labeled with [^{35}S]methionine for 5 hr. Lanes: 1 and 2, immunoprecipitates from untreated cell extracts using anti-Ia.W39 or anti-$I\text{-}A^b$ serum, respectively; 3 and 4, immunoprecipitates from extracts of cells treated with tunicamycin at 2 μg/ml; 5 and 6, immunoprecipitates of cells treated with tunicamycin at 5 μg/ml. Immunoprecipitates using anti-Ia.W39 serum are in lanes 3 and 5, and immunoprecipitates using anti-$I\text{-}A^b$ serum are in lanes 4 and 6. These precipitates were run on a discontinuous 7–15% $NaDodSO_4$, linear acrylamide gradient, which splits the normal two-band Ia pattern into three bands. The middle band corresponds to the Ii chain.

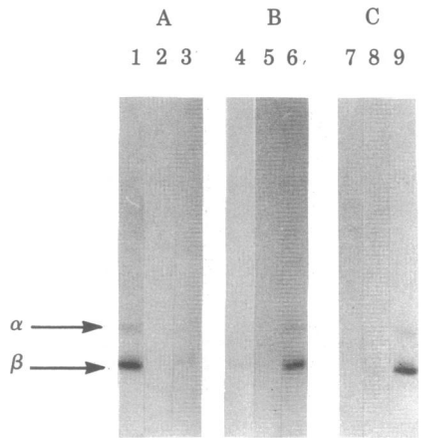

FIG. 5. Autoradiographs of NaDodSO$_4$/polyacrylamide gel electrophoresis of Ia.W39- and conventional Ia-associated polypeptides analyzed by sequential immunoprecipitation experiments. B6 spleen cells were metabolically labeled with [^{35}S]methionine for 24 hr. (*A*) Clearing with anti-*I*-A^b serum. Lanes: 1, immunoprecipitate using anti-*I*-A^b serum; 2 and 3, immunoprecipitates from the resulting supernatant, using either anti-Ia.W39 (lane 2) or anti-*I*-A^b serum (lane 3). (*B*) Clearing with anti-Ia.W39 serum. Lanes: 4, immunoprecipitate using anti-Ia.W39 serum; 5 and 6, immunoprecipitates from the resulting supernatant, using either anti-Ia.W39 (lane 5) or anti-*I*-A^b serum (lane 6). (*C*) Control experiment. Lanes: 7, immunoprecipitate using NMS; lanes 8 and 9, immunoprecipitates from the resulting supernatant, using either anti-Ia.W39 (lane 8) or anti-*I*-A^b serum (lane 9). Indicated bands are as described in the legend to Fig. 1.

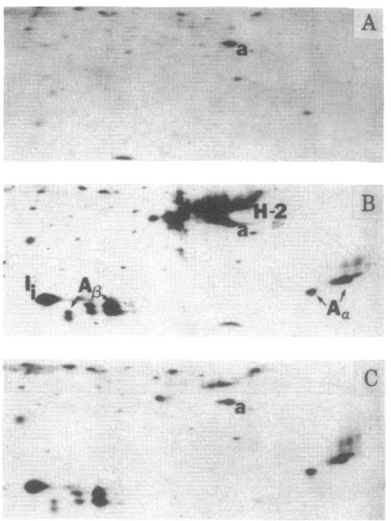

FIG. 6. Two-dimensional polyacrylamide gel electrophoresis analysis of immunoprecipitated Ia antigens. Proteins extracted from 10^7 [^{35}S]methionine-labeled C57BL/10 spleen cells were immunoprecipitated with 25 μl of normal mouse serum (*A*), C3H anti-C3H.SW (anti-*H-2*^{b}) (*B*), or anti-Ia.W39 (*C*). Samples consisted of 40% of each immunoprecipitate. The charge separation was from right (pH 4.5) to left (pH 8.5). The size separation was from top to bottom; the portions of the gel shown include proteins migrating with apparent M_r of 52,000–26,000. Spots corresponding to actin (a), H-2, and Ia chains A_α, A_β, and Ii (16) are indicated. The left-hand (lower molecular weight, more basic) of the A_α and A_β species represent cytoplasmic precursor forms; the right-hand (higher molecular weight, more acidic) of the A_α and A_β species represent all surface forms.

and β chains of the two immunoprecipitates (cytoplasmic and all surface species) were identical in charge and size. These results suggest that Ia.W39 is present on a subset of molecules containing normal A_α^b, A_β^b, and Ii chains.

DISCUSSION

The fact that Ia.W39 is a private specificity of *I*-A^b and is selectively expressed on a functionally defined subset of B cells and macrophages makes it an interesting antigen for structural studies. We have already determined its unequivocal role in the optimal presentation of beef insulin, an antigen that is under Ir control mapping in the *I*-A^b region (27), to immune T cells. These experiments suggest that Ia.W39 is a specific Ir gene product.

The main conclusions drawn in this paper are that the molecule expressing the B-cell-differentiation antigen Ia.W39 has a two-chain structure similar to that expressing the classical Ia specificities; however, it represents a separate group of molecules whose cell surface expression is controlled by *xid*, a regulatory gene on the X chromosome, which has no influence on conventional Ia. The evidence for two sets of molecules possessing A_α^b and A_β^b chains comes from several experiments: (*i*) the glycoprotein bearing the Ia.W39 specificity appears later in ontogeny than that bearing conventional Ia specificities; (*ii*) its membrane expression is controlled by a gene on the X chromosome; (*iii*) its polypeptides turn over more slowly; and (*iv*) the intensity of immunoprecipitation with anti-Ia.W39 serum is weaker, indicating that there are fewer molecules carrying Ia.W39 than classical Ia specificities.

As we had shown previously by cytotoxicity experiments that the gene coding for Ia.W39 maps within the *I*-A^b subregion (22), we wanted to confirm this by immunoprecipitation experiments, using the H-2^b recombinant mice B10.A(5R) (*I*-A^b) and B10.A(4R) (*I*-A^k). The results were clearcut: Only from spleen cells of the B10.A(5R) mice were specific bands precipitated by the anti-Ia.W39 serum. It is worthwhile to stress the unique specificity of the anti-Ia.W39 serum for *I*-A^b; no crossreaction could be found with any other known H-2 type, both by cytotoxicity (22) and by immunoprecipitation studies (results not shown). Furthermore, the bands immunoprecipitated by anti-Ia.W39 serum comigrate with the α and β chain, the two noncovalently linked glycoproteins of the conventional Ia molecule. Therefore, Ia.W39 has the apparent structure of a classical Ia antigen. However, in later studies, we found that the α chain of the Ia.W39 molecule is synthesized at a lower rate than the α chain of the conventional Ia molecule (Fig. 3). These results suggest that there may be two kinds of Ia molecules, depending on the subset of B cells on which they are expressed: Both molecules would bear all the conventional Ia specificities, but one has a relatively fast rate of synthesis (less mature B-cell subset) and the other has a relatively slow rate of synthesis (more mature B-cell subset). The latter subset would include those expressing the Ia.W39 specificity. This is the most likely possibility, considering the identical two dimensional gel picture obtained with the specific Ia.W39 and conventional Ia immunoprecipitates.

Ia.W39 is expressed on 50% of splenic B cells from normal mice and is absent from bone marrow cells and B cells of *xid*-defective mice. Thus, we analyzed its appearance during ontogeny. By using cytotoxicity experiments, Ia.W39 could be detected on spleen cells of ≈3-week-old mice, at the same time when Lyb3, another B-cell-differentiation antigen, is expressed (24). However, when immunoprecipitations from metabolically labeled whole spleen cell lysates were used, Ia.W39 could be detected much earlier, at ≈8 days of age. There seems to be a considerable time span from the moment that Ia.W39 is synthesized in the cytoplasm until it is assembled in the B-cell membrane. Conventional Ia, on the other hand, could be detected on newborn spleen cells both by cytotoxicity and by immunoprecipitation. These results indicate that there are two kinds of Ia molecules containing A_α and A_β chains on spleen cell

membranes. The same time course of appearance seems to take place in macrophages; we found that macrophages of neonatal (up to 10-day-old) B6 mice were able to present trinitrophenylated ovalbumin, but not beef insulin, to immune T cells. The expression of conventional Ia on antigen-presenting cells is sufficient for the former antigen, although Ia.W39 is essential for the latter (27).

A late addition of a new antigenic marker on a normal Ia molecule could be explained easily, if it would be a carbohydrate specificity. To test this, we used the antibiotic tunicamycin, which inhibits N-linked glycosylation. However, previous treatment with this drug did not interfere with the specific immunoprecipitation by either anti-Ia.W39 or anti-Ia^b serum. The constant shift in apparent molecular weight after tunicamycin treatment at various doses (2–5 μg) is proof that N-linked glycosylation was inhibited completely. These studies do not rule out the possibility of O-linked glycosylation; however, this is rarely found on membrane proteins.

By using conventional alloantisera, five specificities (Ia.3, .8, .9, .15, and .20) have been described, all coded for within the $I\text{-}A^b$ subregion. Apparently, they all exist on the same molecule, as they are coprecipitable (37). The fact that only anti-Ia^b serum could preclear Ia.W39 but not vice versa can be interpreted in a number of ways. The most likely explanation is that all molecules bear the conventional specificities, but a subset expresses the Ia.W39 antigen. The allo-anti-$I\text{-}A^b$ serum could therefore also contain antibodies directed against Ia.W39. Consistent with this idea is the finding that anti-$I\text{-}A^b$ serum can block T-cell proliferation to beef insulin to the same extent as anti-Ia.W39 serum, although only the Ia.W39 specificity is associated with this Ir gene (27). Another possible explanation involved a posttranslational modification of Ia that would take place in mature B cells, leading to the appearance of the Ia.W39 specificity. The tunicamycin experiment, however, suggests that this posttranslational alteration is not an N-linked glycosylation.

The data obtained in the two-dimensional gel analysis are compatible with two models. (*i*) The molecules bearing Ia.W39 or conventional specificities (or both) are encoded by the same gene(s) and have identical chains, but are folded differently due to interaction with other membrane determinants that are controlled by *xid*—e.g., Lyb3, 5, or 7. (*ii*) The two molecules differ at uncharged amino acids and, therefore, are products of separate, maybe duplicated, genes. This would imply an extensive polymorphism within the $I\text{-}A^b$ molecule species. Another possibility should also be considered; namely, that Ia.W39 is associated with a different molecule (e.g., glycolipid or small protein) that binds noncovalently to $I\text{-}A^b$.

We would like to thank Dr. Martin Dorf for his generous gifts of H-2 recombinant mice and anti-Ia sera. The expert technical assistance of Ms. Sherborne Abbott and Ms. Patricia Busto-Lattore is acknowledged. This work was supported by National Institutes of Health Grants AI-14910, CA-24530, AI-15310, and AI-15732.

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