GUINEA PIG IMMUNE RESPONSE-RELATED HISTOCOMPATIBILITY ANTIGENS

Partial Characterization and Distribution

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Immune responses to a series of specific antigens are controlled by a group of immune response (*Ir*)[1] genes linked to the major histocompatibility complex (MHC) of the species (1, 2). Thus, in inbred guinea pigs responsiveness to several antigens, including the 2,4-dinitrophenyl (DNP) derivatives of poly-L-lysine, the DNP derivative of the copolymer of L-glutamic acid and L-lysine (GL) and the copolymer of L-glutamic acid and L-alanine (GA), are controlled by *Ir* genes linked to the strain 2 MHC while responsiveness to the copolymer of L-glutamic acid and L-tyrosine (GT) is linked to the strain 13 MHC. We have recently demonstrated that alloantisera produced by immunizing strain 13 guinea pigs with strain 2 lymphoid tissue will block in vitro responses of primed $(2 \times 13)F_1$ thymus-dependent lymphocytes to DNP-GL and GA, and not to GT (3). Alloantisera with specificity for strain 13 alloantigens block responsiveness of $(2 \times 13)F_1$ cells to GT, but not to DNP-GL or GA (4). This indicates that such alloantisera in some way specifically block the function of the *Ir*-gene product and suggests an important role for that gene product in antigen recognition by T lymphocytes.

An analysis of the relative capacity of anti-2 alloantisera to block responsiveness to GA of cells from outbred guinea pigs which are 2^+GA^+ and those which are 2^-GA^+ strongly suggests that the inhibitory antibodies are directed at the "2" alloantigen rather than at the product of the linked *Ir* gene (3). This finding raises the possibility that the alloantigen and *Ir*-gene product, although products of independent genes, may be physically linked on the cell surface.

In order to evaluate the mechanism of blockade by alloantiserum and the molecular basis for *Ir*-gene product function, we have initiated studies of the chemical nature of the antigens with which the anti-2 and anti-13 sera interact. In addition, we have also studied an alloantigen encoded at a distinct, but very likely, linked genetic locus. This antigen, "B," is a member of a family of at least three antigens (B, C, and D) that was initially

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[1] *Abbreviations used in this paper:* GA, copolymer of L-glutamic acid and L-alanine; GL, copolymer of L-glutamic acid and L-lysine; GT, copolymer of L-glutamic acid and L-tyrosine; Ia, I-region-associated antigen; *Ir* gene, immune response gene; MHC, major histocompatibility complex; NP-40, Nonidet P-40; PBS, phosphate-buffered saline; PELs, peritoneal exudate lymphocytes; SDS-PAGE, SDS-polyacrylamide electrophoresis.

described by Sato and de Weck (5). Both strain 2 and strain 13 guinea pigs are B^+, C^-, and D^-. In our hands, responses of cells from 2, 13, and (2 × 13)F_1 guinea pigs to DNP-GL, GA, GT, and purified protein derivative of tuberculin are not specifically inhibited by anti-B sera, although this serum has a general inhibitory effect on responses of B^+ cells to antigens and phytomitogens. Moreover, immunofluorescence studies indicate that the B antigen has a wide tissue distribution whereas the 2 and 13 antigens are largely limited to lymphoid cells (6). Thus, the B antigen provides an interesting contrast in the study of the structure of the 2 and 13 antigens.

In this communication, we report results of the analysis of antigens isolated from the surface of lymphoid cells of inbred guinea pigs. Lactoperoxidase-catalyzed iodination was used to selectively label cell surface antigen. After iodination, the antigens were extracted from the cells with a nonionic detergent, precipitated with specific antisera, and subjected to electrophoresis on polyacrylamide-sodium dodecyl sulfate gels. Our results indicate that the 2 and the 13 antigens each behave as single polypeptide chains with mol wt of approximately 25,000. The B alloantigen, on the other hand, is composed of two types of molecules, one with a mol wt of about 45,000 and the other with a mol wt of about 12,000.

Materials and Methods

Animals. Inbred strain 2 and strain 13 guinea pigs were obtained from the Division of Research Services, National Institutes of Health, Bethesda, Md.

Preparation of Antisera. Strain 13 antistrain 2 and strain 2 antistrain 13 antisera were prepared as previously described (4). Antisera to the B, C, and D alloantigens were a gift of Dr. A. de Weck, Inselspital, Berne, Switzerland. Hartley guinea pigs were screened for the presence of 2, 13, B, and C antigens by ^{51}Cr cytotoxicity testing with the appropriate antisera. A $2^-13^+B^-C^+$ animal was identified, immunized with inbred strain 13 ($2^-13^+B^+C^-$) lymph node and spleen cells in complete Freund's adjuvant, and boosted three times by intraperitoneal injection of saline suspensions of strain 13 cells. The resultant antiserum was highly cytotoxic for both inbred strain 2 and strain 13 lymph node cells. All of the cytotoxic activity of this antiserum for strain 13 lymph node cells could be absorbed out with strain 2 cells. This sera is regarded as principally specific for the B alloantigen and typing of a large number of outbred guinea pigs is consistent with this assumption.

A rabbit antiguinea pig immunoglobulin antiserum was prepared by immunizing rabbits with electrophoretically pure guinea pig IgG. A sheep antirabbit immunoglobulin antiserum was a generous gift of Dr. John Robbins (National Institutes of Health, Bethesda, Md.).

Preparation of IgG Fractions of Antisera. In order to reduce possible artifacts generated by nonspecific binding of cell membrane proteins to serum proteins, purified IgG fractions of all antisera were prepared. 40% saturated ammonium sulfate precipitates of each antiserum were washed in 40% saturated ammonium sulfate, then dissolved in water and dialyzed against 0.1 M Tris buffer at pH 8.2. Dialysates were applied to columns of DE-52 (W & R Balston, Ld., Maidstone, England) in 0.1 M Tris (pH 8.2) and eluted with the same buffer. Column eluates contain highly purified IgG, as demonstrated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Eluates were concentrated with an Amicon ultrafiltration apparatus, using Diaflo UM-10 filters (Amicon Corp., Lexington, Mass.). IgG fractions of anti-2, anti-13, and anti-B antisera were shown to be cytotoxic for appropriate lymphocytes by the ^{51}Cr-release assay. IgG fractions of anti-2 and anti-13 antisera inhibited in vitro immune responses in the same manner as the antisera from which they were derived.

Cell Collection and Purification. Peritoneal exudate lymphocytes (PELs), lymph node cells, and L_2C leukemia cells were prepared and purified as previously described (7). The L_2C leukemia appeared spontaneously in strain 2 guinea pigs in 1954. Tumor cells were given to us by Dr. L. Kaplow

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of Yale University, New Haven, Conn., and have been carried in our laboratory by serial transfer. By a variety of criteria, L_2C is a leukemia of B lymphocytes (8).

Surface Iodination of Lymphoid Cells. Cell membrane proteins were ^{125}I labeled by the lactoperoxidase technique of Phillips and Morrison (9) as modified by Vitetta et al. (10). Briefly, 2–10 × 10^7 cells in 2 ml of phosphate-buffered saline (PBS) were mixed with 2 mCi of $Na^{125}I$ (New England Nuclear, Boston, Mass.) and 0.1 mg of lactoperoxidase (Calbiochem., San Diego, Calif.) at room temperature. Two 25-μl portions of 0.015% hydrogen peroxide in PBS were added at 5-min intervals. After 10 min, the reaction was stopped by the addition of 50 ml of ice-cold PBS, and the labeled cells washed twice in PBS. The washed cells were then treated for 10 min in 2 ml of 0.5% Nonidet P-40 (NP-40) (Particle Data, Inc., Elmhurst, Ill.). Cellular debris were removed by centrifugation at 3,000 rpm for 15 min. 10 μl of a 1 M solution of diisopropylfluorophosphorophosphate (Calbiochem) were added to the centrifugate to inhibit enzymatic breakdown of labeled proteins. The centrifugate was then dialyzed against PBS. All procedures subsequent to ^{125}I labeling were performed at 0–4°C.

Immunoprecipitation of Cell Membrane Antigens. ^{125}I-labeled cell membrane antigens were precipitated from cell lysates using an antibody "sandwich" technique. Fig. 1 schematically depicts the procedure for the precipitation of 2 and B antigens from lysates of strain 2 cells. Steps 1 and 2 in the scheme were performed to remove membrane-bound immunoglobulin (Ig) as well as other labeled molecules binding nonspecifically to the complex. In these steps, rabbit antiguinea pig Ig was added and was precipitated with an equivalence amount of sheep antirabbit Ig. In all subsequent steps Ig isolated from alloantisera was added and precipitated with equivalence amounts of rabbit antiguinea pig Ig. Precipitate 3 is a negative control, as a 2 anti-13 antiserum is not expected to specifically bind

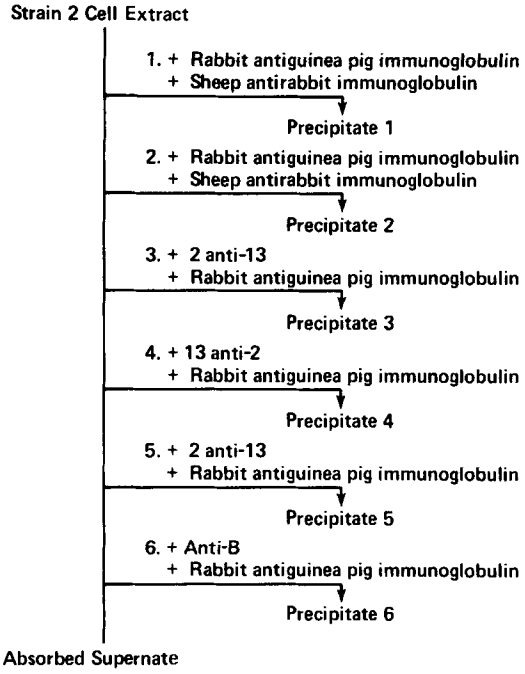

FIG. 1. Procedure used to precipitate the 2 and B antigens from an NP-40 extract of radioiodinated strain 2 cells. Precipitations 1 and 2 remove ^{125}I-labeled cell membrane-bound Ig from the extract. Precipitate 3 is a negative control. Precipitate 4 is specific for the ^{125}I-labeled cell membrane antigens bound by 13 anti-2 antibody. Precipitation 5 removes soluble antigen-antibody complexes containing ^{125}I-labeled 2 antigen remaining after precipitation 4. Precipitate 6 is specific for the ^{125}I-labeled cell membrane antigen bound by anti-B antibody.

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cell membrane proteins of strain 2 cells. Precipitates 4 and 6 contain those cell membrane antigens recognized by 13 anti-2 and anti-B antibodies, respectively. Step 5 was performed to remove any soluble antigen-antibody complexes containing 2 antigen remaining after step 4. The same precipitation schema was used in experiments in which cell membrane proteins were precipitated from lysates of strain 13 cells, with the exception that steps 3 and 5 were performed with 13 anti-2 antiserum, and step 4 with 2 anti-13 antiserum.

SDS-PAGE of Precipitates Containing Cell Membrane Antigens. Precipitates were washed extensively in PBS, then dissolved by boiling in 50–100 μl of 2% SDS for 2 min. In some experiments, 50 μl of 2-mercaptoethanol were added to reduce disulfide bands. The Laemmli system of discontinuous SDS-PAGE was used to resolve labeled molecules (11). Samples were applied to both 11% and 15% acrylamide gels to maximize resolution of molecules in the mol wt range of 10,000–100,000. Gels were electrophoresed in a PAGE cell purchased from Pharmacia Fine Chemicals, Inc., Piscataway, N. J. Peaks representing immunoprecipitated ^{125}I-labeled cell membrane antigen were identified by dividing gels into 1-mm fractions with a Gilson gel mincer (Gilson Medical Electronics, Inc., Middleton, Wis.), and counting fractions in a Picker gamma counter (Picker Corp., Cleveland, Ohio). RNase A and guinea pig Ig μ-, γ-, and L-chains were electrophoresed concurrently with labeled cell membrane antigens as molecular weight markers.

Results

Lymph node cells (5–10 × 10^7) from strain 2 guinea pigs were iodinated by the lactoperoxidase procedure and surface molecules extracted and analyzed as outlined in the Materials and Methods. NP-40 extracts of most labeled cell preparations had activities of approximately 10^7 cpm, of which 40–50% were TCA precipitable. Initial immunoprecipitation with anti-Ig revealed the presence of both IgM and IgG as indicated by SDS-PAGE (data not shown). This precipitation step was repeated to insure complete removal of ^{125}I-labeled Ig. Completeness of removal of such Ig is demonstrated by the electrophoresis of the next precipitate, prepared with a 2 anti-13 antibody and rabbit antiguinea pig Ig. No specific peaks are observed upon electrophoresis of the dissolved anti-13 precipitate although variable amounts of radioactivity are found at the origin and at the electrophoretic front (Fig. 2). We have not analyzed this material but it presumably reflects large aggregates, iodinated lipid, and enzymatically degraded material (10). Following this, a precipitate was prepared with a 13 anti-2 antibody and rabbit antiguinea pig Ig. SDS-PAGE analysis of this immunoprecipitate reveals a single distinctive radioactive peak with a mobility slightly slower than that of L chains. This mobility is consistent with a mol wt of about 25,000. Addition of 2-mercaptoethanol to the SDS solution of 2 alloantigen does not change the mobility of this material on subsequent SDS-PAGE, indicating that it is not composed of smaller polypeptide chains cross linked by disulfide bonds.

When the remaining supernate is precipitated with anti-B alloantibody and antiguinea pig Ig antibody, the material obtained migrates as two principal radioactive peaks on SDS-15% polyacrylamide gels. One peak has a mobility consistent with a mol wt of about 45,000, and the second peak, which always contains less radioactivity, has a mobility consistent with a mol wt of about 12,000. The latter peak is not detected on SDS–11% polyacrylamide gels, presumably because it migrates with the electrophoretic front. In some runs, a third peak representing a mol wt of about 56,000 is observed. This peak can be

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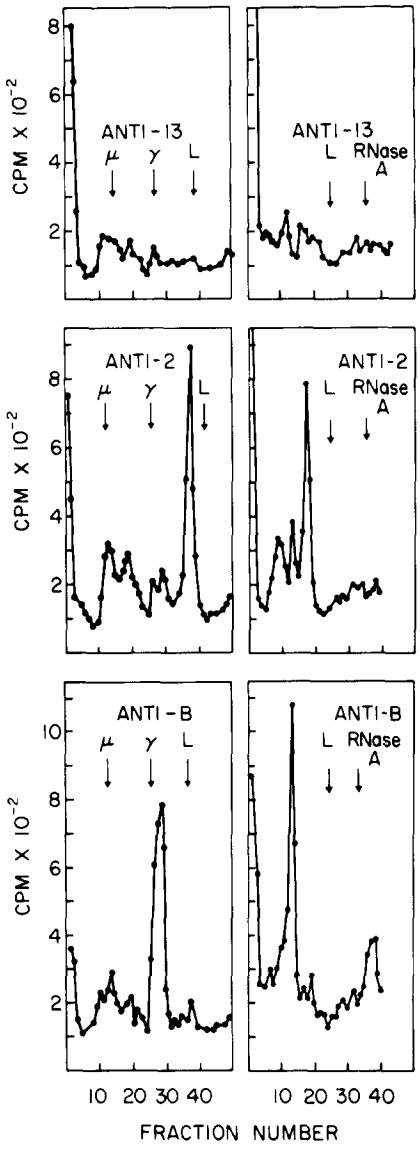

FIG. 2. Acrylamide gel electrophoresis of 2 and B molecules from strain 2 lymph node cells. Membrane antigens from strain 2 lymph node cells were enzymatically radioiodinated, extracted with NP-40, and immunoprecipitated as described in Fig. 1. Precipitates were dissolved in 2% SDS, and electrophoresed on SDS 11% acrylamide gels (left) and SDS 15% acrylamide gels (right). No antigen is specifically precipitated with 2 anti-13 antiserum (negative control, top panel), while the 13 anti-2 precipitate (middle panel) yields a single peak representing a mol wt of about 25,000. The anti-B precipitate (bottom panel) yields two peaks, corresponding to mol wt of approximately 45,000 and 12,000. These molecular weights are determined by the positions of peaks relative to the positions of markers of known molecular weights (γ = 55,000; L = 22,000; RNase A = 13,700).

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eliminated by increasing the length of time the anti-B immunoprecipitate is boiled in SDS. It may represent an aggregate of the two major B peaks, but no direct evidence demonstrating this has yet been obtained. Reduction of B with 2-mercaptoethanol does not change the mobility of either of the principal peaks suggesting that the B antigen does not consist of polypeptide chains linked by disulfide bonds.

Fig. 3 depicts the results obtained when anti-2, anti-13, and anti-B precipitates from strain 13 lymph node cell extracts were electrophoresed on 11% and 15% gels. It can be seen that the anti-2 precipitate yields no peak, while the anti-13 precipitate yields a single peak which migrates identically to the peak obtained from an anti-2 precipitate of a strain 2 lymph node cell extract. This strengthens the contention that the 2 and 13 genes are alleles at a single locus or at a series of loci that code for products of the same molecular size. With regard to the amount of radioactivity they contain, 13 antigen peaks derived from strain 13 lymph node cells are similar to 2 antigen peaks derived from strain 2 lymph node cells. The anti-B precipitate yields the same two peaks seen with anti-B precipitates from a strain 2 lymph node cell extract. This result is anticipated from the finding that strain 2 and strain 13 guinea pigs are both B^+.

Serologically, 2 and 13 antigens are detectable on both bone-marrow-derived (B) and thymus-dependent (T) lymphocytes. Studies involving relative ease of cytolysis and relative absorptive capacity have suggested that the 2 and 13 alloantigens are more heavily represented on B lymphocytes than T lymphocytes (7). In order to study the tissue distribution of these antigens more completely, we subjected cell populations enriched in B and T lymphocytes to the surface-labeling procedure and analyzed the resultant labeled histocompatibility antigens. As a source of B lymphocytes, we chose L_2C leukemia cells. The latter is a strain 2 leukemia which bears surface Ig and receptors for the third component of complement, and is thus classified as a B-cell leukemia. By absorption tests, this cell is relatively rich in the 2 alloantigen (12). As a source of cells enriched in T lymphocytes, we chose PELs purified from exudates of strain 2 and strain 13 guinea pigs.

L_2C cells were obtained from the peripheral blood of leukemic strain 2 guinea pigs which had leukocyte counts of greater than 300,000/mm^3. These cells were iodinated in the usual way and the extract analyzed by sequential immunoprecipitation and SDS-PAGE on 12 and 16% gels (Fig. 4). Both 2 and B antigens were present and they had molecular weights indistinguishable from those of antigens extracted from the strain 2 lymph node cell population. As anticipated, no peak was precipitated from the L_2C extract with anti-13 serum. In view of the fact that L_2C cells are a very large fraction of peripheral blood cells in the leukemic guinea pigs, it seems extremely likely that these results describe molecules present on the L_2C cells and not on some other cell type contaminating the preparation.

PELs, which were used as an enriched source of peripheral T lymphocytes, in fact contain fewer than 5% Ig-bearing lymphocytes. These cells are also contaminated by variable numbers of macrophages (up to 15%) (13). PELs from strain 2 and strain 13 guinea pigs were iodinated and analyzed as above. Again,

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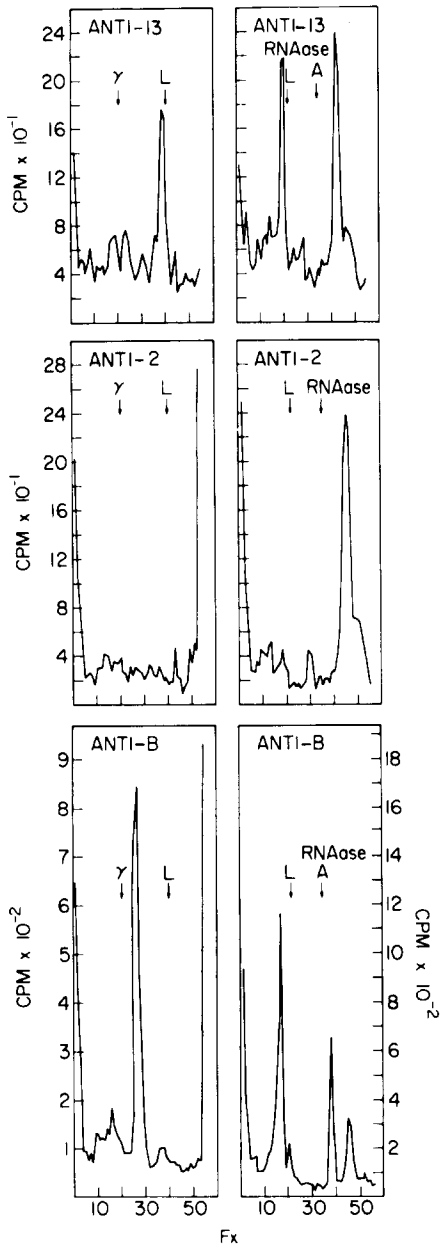

FIG. 3. Acrylamide gel electrophoresis of 13 and B molecules from strain 13 lymph node cells. 2 anti-13, 13 anti-2, and anti-B precipitates were prepared from an NP-40 extract of enzymatically radioiodinated strain 13 lymph node cells and electrophoresed on SDS 11% acrylamide gels (left) and SDS 15% acrylamide gels (right). The 2 anti-13 precipitate (upper panel) yields a single peak corresponding to a mol wt of about 25,000, the 13 anti-2 precipitate (negative control) does not yield a peak (middle panel), and the anti-B precipitate yields two peaks corresponding to mol wt of about 45,000 and 12,000 (lower panel).

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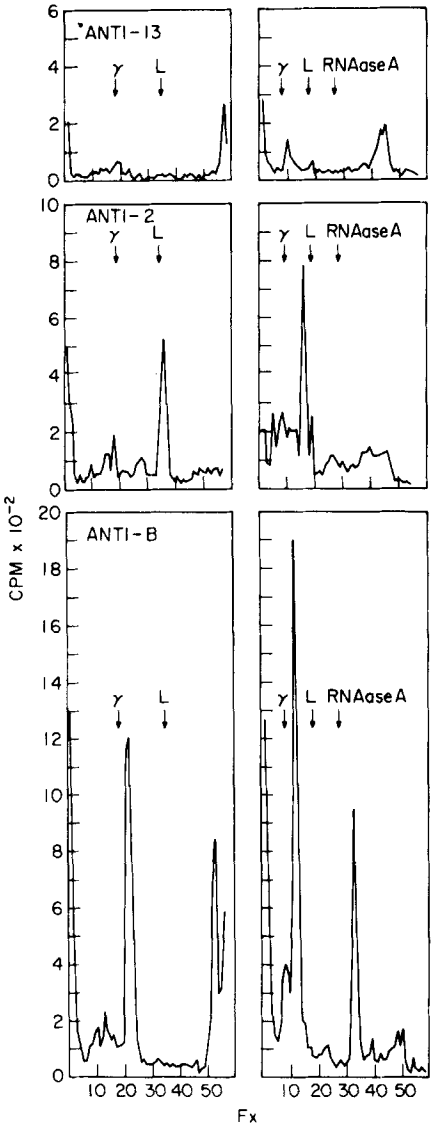

FIG. 4. Acrylamide gel electrophoresis of 2 and B molecules from L_2C leukemia cells. 2 anti-13, 13 anti-2, and anti-B precipitates were prepared from an NP-40 extract of radioiodinated L_2C cells, and electrophoresed on SDS 12% acrylamide gels (left) and SDS 16% acrylamide gels (right). The 2 anti-13 precipitate (negative control) yields no peak (upper panel); the 13 anti-2 precipitate (middle panel) yields a single peak corresponding to a mol wt of approximately 25,000, and the anti-B precipitate (lower panel) yields two peaks corresponding to mol wt of about 45,000 and 12,000.

both 2 and B antigens were isolated from strain 2 PELs (Fig. 5) and 13 and B antigens were isolated from strain 13 PELs (Fig. 6). By SDS-PAGE, these antigens were indistinguishable from those isolated from lymph node cells. In all cases, both the 45,000 and 12,000 mol wt peaks were observed when precipitates

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obtained with anti-B sera were analyzed on SDS–15% polyacrylamide gels (data not shown).

Because of the presence of small numbers of B lymphocytes and larger numbers of macrophages in the PEL preparation, the possibility remained that the 2 and 13 antigens might derive from these cells rather than from the T lymphocytes, which are the principal cells present in this population. However, iodination of unfractionated peritoneal exudate cells yields large B peaks but no detectable 2 or 13 peaks. As the purification procedure involved in preparing PELs from peritoneal exudates tends to reduce the numbers of macrophages, polymorphonuclear leukocytes, and B lymphocytes, the presence of substantial 2 and 13 peaks in extracts of PELs but not in peritoneal exudate cell extracts can hardly be a function of the non-T cells in PEL preparations. Thus it seems certain that the 2 and 13 antigens (as well as B) isolated from PELs represents T-lymphocyte antigens and that by size criteria 2 and B antigens extracted from B and T lymphocytes are not distinguishable.

Discussion

In this study we have demonstrated that anti-2 and anti-13 antisera each recognize a single cell membrane antigen or class of cell membrane antigens with mol wt of approximately 25,000. Anti-B antiserum recognized either two cell membrane antigens with mol wt of approximately 45,000 and 12,000 or a single cell membrane antigen composed of noncovalently linked 45,000 and 12,000 mol wt components. The occasional finding on SDS gels of a peak representing a mol wt of approximately 56,000 which is lost upon further boiling in SDS makes the second possibility more likely. It should, of course, be noted that since the 2, 13, and B antigens are glycoproteins, the molecular weights determined by SDS-PAGE may be overestimates.

The finding that B and 2 determinants are independently precipitated from NP-40 extracts indicates that they are present on separate cell membrane molecules. This has been confirmed by immunofluorescent-capping experiments performed by us on strain 2 PELs. These experiments indicated that after capping the 2 antigen with anti-2 antiserum followed by rabbit antiguinea pig Ig antiserum, the B antigen could still be identified on the periphery of the cell membrane, and that if B were capped first, 2 could still be identified on the cell membrane periphery (6). By labeling populations of cells enriched in B lymphocytes (malignant L_2C cells) and populations enriched in T cells (PELs) we obtained evidence that the strain 2 alloantigen is present on both B and T lymphocytes. While we have previously demonstrated the presence of the 2 and 13 alloantigens on peripheral T lymphocytes in the peritoneal exudate by indirect immunofluorescence, quantitative absorption studies indicated that PELs are much less efficient than L_2C cells or lymph node cells in removing anti-2 antibody (7). This led us to conclude that T lymphocytes bear less 2 antigen than B lymphocytes. In surface-labeling studies, we did not observe any obvious quantitative difference in the amount of radioactivity incorporated into the 2 antigen in PELs or L_2C-cell preparations. Differences in the completeness of

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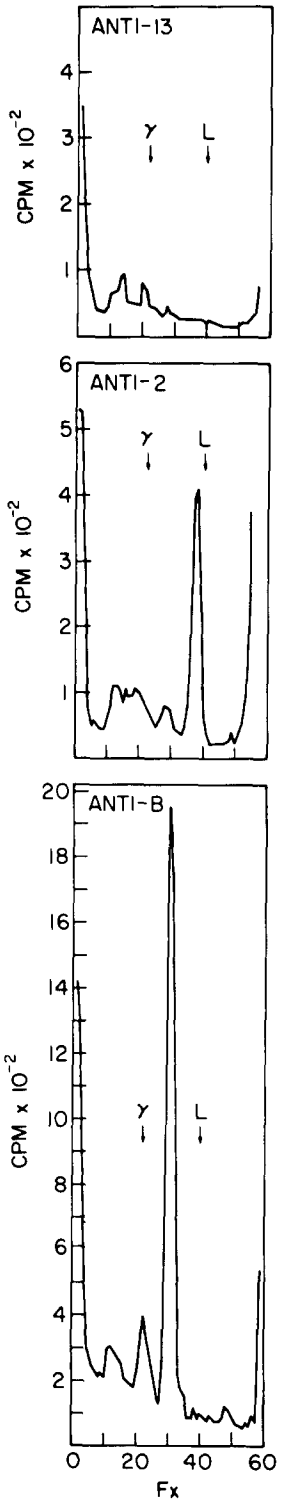

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^{125}I-labeled lactoperoxidase labeling and detergent extraction of the antigen from T- and B-lymphocyte membranes are possible, and may account for this apparent discrepancy. Alternatively, B and T lymphocytes might have similar amounts of the 2 antigen, but the positioning of the 2 antigen on B- and T-lymphocyte cell membranes may differ, so that the determinant bound by anti-2 antibody may be more available on B lymphocytes than on T lymphocytes.

One finding of considerable interest is that the 2, 13, and B antigens extracted from populations rich in T lymphocytes have identical mobilities on SDS-PAGE as those antigens extracted from mixed T- and B-lymphocyte populations and in the case of 2 and B, from B-lymphocyte leukemia cells. This indicates that the molecular weight of these alloantigens is the same on T and B lymphocytes (14).

Both the 2/13 and B antigens seem to be homologous to human and mouse alloantigens. The B antigen closely resembles both the mouse D and K and human LA and Four antigens with respect to molecular weight (15,16). The larger peptides of these antigens have been found to have mol wt of approximately 45,000 for the mouse and guinea pig, and 43,000 for humans, and all three antigens have a low mol wt peptide of approximately 12,000, which, for humans, has been identified as β2-microglobulin (17). This suggests that the B antigen, rather than the 2/13 antigens, is homologous to the human and mouse major "serologically-defined" histocompatibility antigens. This suggestion is supported by recent immunofluorescence data, which identify the B but not the 2/13 antigens on a carcinogen-induced hepatoma cell and a virus-induced fibrosarcoma, thus indicating that the widespread distribution of the human LA and Four and mouse K and D antigens is more closely matched by the B antigen than by the 2/13 antigens (6).

By both molecular weight and distributional criteria, the 2/13 antigens seem to resemble the I-region-associated (Ia) antigens of mice. These alloantigens, coded for by cistrons in the *Ir* region of the mouse MHC, have been found by two groups to have mol wt of about 30,000 (18,19). These antigens are also similar to the 2/13 antigens in that they appear to be restricted to lymphoid cells and a few other cell types (20). Some antisera identify them only on B lymphocytes; other antisera identify them both on B and T lymphocytes (21).

The finding that the 2 and 13 antigens have similar molecular weights is consistent with the idea that they represent products of allelic genes. Whether there is a single 2 or 13 gene or a series of linked genes each encoding a molecule of the same size, but of different primary structure is unknown. It may be possible to determine this by absorption of anti-2 and anti-13 sera with cells from individual outbred guinea pigs. If one could demonstrate that cells from some outbred animals absorb some but not all anti-2 (or anti-13) antibodies, and that

FIG. 5. Acrylamide gel electrophoresis of 2 and B molecules from strain 2 PELs. PELs are a largely lymphocyte population, of which greater than 90% of lymphocytes are T lymphocytes. 2 anti-13, 13 anti-2, and anti-B precipitates were prepared from an NP-40 extract of radioiodinated strain 2 PELs and electrophoresed on SDS 11% acrylamide gels. The 2 anti-13 precipitate (negative control) yields no peak (upper panel). The 13 anti-2 precipitate (middle panel) yields a single peak corresponding to a mol wt of about 25,000. The anti-B precipitate yields a single peak corresponding to a mol wt of about 45,000.

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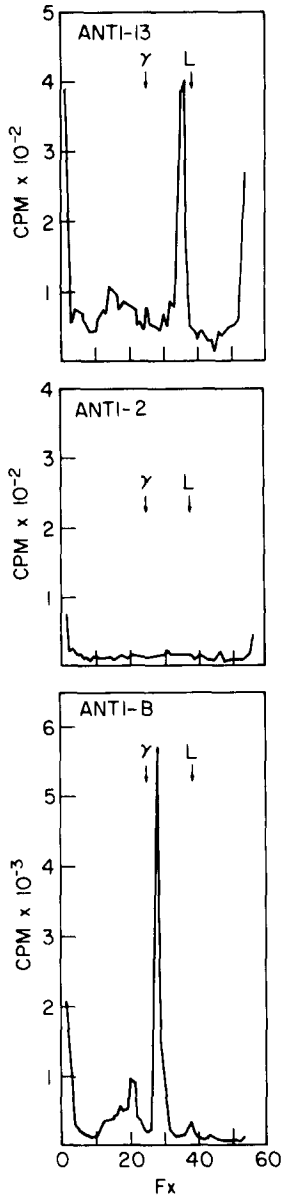

FIG. 6. Acrylamide gel electrophoresis of 2 and B molecules from strain 13 PELs. 2 anti-13, 13 anti-2, and anti-B precipitates were prepared from an NP-40 extract of radioiodinated strain 13 PELs and electrophoresed on SDS 11% acrylamide gels. The 2 anti-13 precipitate yields a single peak corresponding to a mol wt of about 25,000 (top panel). The 13 anti-2 precipitate (negative control) yields no peak (middle panel). The anti-B precipitate yields a single peak corresponding to a mol wt of about 45,000 (bottom panel).

such absorbed antisera precipitate some but not all molecules bearing 2 (or 13) antigen(s), it would suggest multiple-linked loci.

Our previous analyses of blockade of *Ir*-gene product function by anti-2 sera suggest that the blocking activity is due to antibodies that bind the 2 alloantigen

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rather than individual *Ir*-gene products. To explain this finding, we proposed that the 2 alloantigen and the *Ir*-gene products are linked on the cell membrane as a complex which acts as either the major or an auxillary T-lymphocyte receptor for antigen (3). This linkage could, like the linkage between Ig variable and constant regions, occur before translation with the result that the 2 alloantigen and the *Ir*-gene product are part of a single polypeptide on the cell membrane surface. If this is the case, we would expect the 2 antigen we have isolated from the cell membrane to have a degree of amino acid heterogeneity similar to that found within an Ig heavy- or light-chain subclass.

Alternatively, the proposed bond between 2 alloantigen and *Ir*-gene product might be more labile than the amide linkage which connects the variable and constant regions of Ig light and heavy chains. If the proposed bond connecting 2 alloantigen and the *Ir*-gene product is sufficiently labile to be disrupted by treatment with NP-40, we would expect the antigen we have isolated with anti-2 antisera to contain only the 2 alloantigen part of the postulated 2-*Ir*-gene product complex, and to be homogeneous with respect to amino acid composition.

One objection to the theory that the 2/13 alloantigens are part of a T-lymphocyte antigen receptor is the finding that the amount of these antigens on B-lymphocyte cell membranes is equal to or greater than the amount found on T-lymphocyte cell membranes. Moreover, the size of these alloantigens on B and T lymphocytes appears to be identical. If these alloantigens do not act as auxillary antigen receptors on B lymphocytes, they may function in the interactions between B and T lymphocytes and between different classes of T lymphocytes (*i.e.* "helpers," "suppressors," and "effectors"). This theory is supported by evidence demonstrating that cooperation between T and B lymphocytes and between macrophages and T lymphocytes is restricted to cells syngeneic or semisyngeneic for products of the major histocompatibility region (22–24). While there is not now conclusive evidence in support of any of these postulates, the theory that the *Ir*-gene product has some specific role in antigen recognition and that the 2 alloantigen and *Ir*-gene product are covalently linked has the virtue of being testable through its prediction of 2 antigen heterogeneity.

Summary

We have previously demonstrated that guinea pig alloantisera directed at strain 2 and strain 13 membrane antigens block specific lymphocyte activation in immune response gene-controlled systems. In this communication we describe the partial characterization of the antigens against which these antisera are directed (the 2 and 13 antigens) and, in addition, that of the B antigen which by distribution resembles the human HL-A and mouse *H-2* major histocompatibility antigens. Lymphoid cells from strain 2 and strain 13 guinea pigs were surface labeled with ^{125}I by the lactoperoxidase technique. Nonidet P-40 extracts of these labeled cells were precipitated by sandwiches of strain 2 antistrain 13, strain 13 antistrain 2, or outbred anti-B antisera, followed by rabbit antiguinea pig immunoglobulin antisera. Precipitates were dissolved in sodium dodecyl sulfate (SDS) and electrophoresed on SDS polyacrylamide gels. Radioactive peaks representing the 2 and B-cell membrane antigens were obtained from strain 2

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lymph node cells, as well as from a B-lymphoid cell population (L_2C leukemia cells) and a T-lymphocyte population (strain 2 peritoneal exudate lymphocytes [PELs]). Radioactive peaks representing the 13 and B-cell membrane antigens were obtained from strain 13 lymph node cells and strain 13 PELs. All anti-B precipitates produced two peaks when electrophoresed on SDS polyacrylamide gels; one representing an antigen with a mol wt of approximately 45,000, and one representing an antigen with a mol wt of about 12,000. Both may be components of a single protein. All anti-2 and anti-13 precipitates produced a single peak when electrophoresed on SDS polyacrylamide gels. Both the 2 and 13 antigens were found by this technique to have mol wt of approximately 25,000. By molecular weight criteria, as well as by previously investigated distributional criteria, the B antigen is similar to the human LA and Four antigens, and to the mouse D and K antigens, and the 2 and 13 antigens are similar to the mouse Ia antigens.

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Note Added in Proof. Recent studies from our laboratory (B. Schwartz and F. D. Finkelman, unpublished observations) in which the B antigen is biosynthetically labeled with [^{3}H]leucine and analyzed by an SDS-PAGE system using different buffers than those used in the work reported here yield a mol wt of approximately 38,000. The discrepancy between that value and the one reported in this communication may reflect differences in the relative mobility of glycoproteins in SDS-PAGE systems employing different buffers.

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