T CELL HYBRIDS THAT EXPRESS A V_H IDIOTOPE-RELATED DETERMINANT ON A GLYCOPROTEIN DISTINCT FROM H-2, THY-1, AND LYT-1 MOLECULES*

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Almost 20 years have passed since the recognition that thymus-derived lymphocytes belong to a separate differentiation pathway and do not produce immunoglobulins (1, 2). However, the molecular structure of the antigen-specific receptor on T cells remains an elusive goal necessary for understanding antigen-induced interactions among T cells, B cells, and antigen-presenting cells in the immune response. There is substantial evidence in support of similarity between immunoglobulin (Ig) idiotype (Id) and determinants expressed by antigen-specific T cells. Anti-Id antibodies have been used to either stimulate or inhibit various T cell functions (3–5), and to demonstrate Ig-like Id determinants on antigen-specific T cells (6–9) and the soluble factors they produce (10, 11). The definition of the T cell receptor is complicated by the existence of functionally distinct subsets of T cells, each capable of expressing distinct antigen-specific molecules (12). A number of laboratories have established stable interleukin 2-dependent T lymphocyte clones (13–15) and T cell hybrids derived from fusion between immune T lymphocytes and cells of thymic lymphoma origin (11, 16–18). This approach should result in an increase in the yield of homogenous antigen-binding materials produced by T cells and thereby facilitate the elucidation of the molecular structure of the antigen-specific T cell receptor.

We have developed two mouse monoclonal anti-Id antibodies specific for chicken antibodies to *N*-acetylglucosamine (NAGA)[1] and *p*-amino benzoic acid (PABA). The anti-Id antibodies, termed CId-1 and CId-2, respectively, were found to react with non–antigen-binding V_H determinants (19). The CId-1 antibody reacted by indirect immunofluorescence with a limited number of clones of both chicken B and T cells, whereas the CId-2 antibody reacted

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; FCS, fetal calf serum; FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; HBSS, Hanks' balanced salt solution; LL, lentil lectin; LPS, lipopolysaccharide; NAGA, *N*-acetylglucosamine; PABA, *p*-amino benzoic acid; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; PWM, pokeweed mitogen; RITC, rhodamine isothiocyanate; Strep A, *Streptococcus pyogenes* group A strain J17A4; WGA, wheat germ agglutinin.

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exclusively with Ig expressed by B cells. We have since found that the CId-1 antibody recognizes a conserved determinant expressed by a small subset of BALB/c mouse splenic T cells. Encouraged by this observation, we fused enriched CId-1$^+$ T lymphocytes obtained from Streptococcus A-immune BALB/c mice with the AKR BW 5147 cell line. Among the resulting 72 hybrids were two clones that reacted by indirect immunofluorescence with the CId-1 monoclonal antibody. In this paper, we describe the generation of these T cell hybrids and an initial characterization of their CId-1 determinants.

Materials and Methods

Antisera. The preparation and characterization of the monoclonal CId-1 and CId-2 anti-Id antibodies (IgMκ) have been described (19). The rat monoclonal antibodies to mouse Lyt-1 and Lyt-2, the mouse monoclonal antibodies to mouse I-A^d and I-A^k and monoclonal fluorescein isothiocyanate (FITC)-conjugated anti-Thy-1.2 antibodies were from Becton, Dickinson & Co., Sunnyvale, CA. The mouse anti-I-J^d and I-J^k alloantisera were gifts from Dr. Chella S. David, Mayo Clinic, Rochester, MN. The anti-H-2^d alloantiserum was a gift from Dr. Lori Flaherty, Albany, NY. Affinity-purified goat antibodies specific for mouse Ig isotypes were prepared as described (20).

Immunization. BALB/c mice (H-2^d) were immunized intraperitoneally three times at 5-d intervals with 10^9 heat-killed Streptococcus group A strain J17A4 (Strep A) organisms.

Enrichment of CId-1$^+$ Splenic T Lymphocytes. 3 d after the last immunization, the mice were killed and spleen mononuclear cells were isolated by centrifugation over Ficoll (Pharmacia Fine Chemicals, Piscataway, NY)-Hypaque (Winthrop Laboratories, NY) gradients. To enrich for T lymphocytes, the spleen cell suspension was panned twice on culture dishes (Costar, Data Packaging, Cambridge, MA) precoated with 100 μg/ml of affinity-purified goat anti-mouse Ig (21). To enrich further for CId-1$^+$ T lymphocytes, the nonadherent cells were treated with 200 μg/ml of the CId-1 monoclonal antibody for 30 min at 4°C, washed with phosphate-buffered saline (PBS) supplemented with 5% fetal calf serum (FCS), and panned on dishes precoated with 100 μg/ml of affinity-purified goat antibodies to mouse μ chains. After a 90-min incubation at 4°C, the dishes were washed five times with 5% FCS in PBS and 10 ml of complete RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) supplemented with 15% FCS, 2 mM glutamine, 5×10^{-5} M 2-mercaptoethanol, 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 0.25 μg/ml of Fungizone (Gibco Laboratories, Grand Island, NY) were added to each plate. After a 1-h incubation at 37°C, the adherent cells were recovered with a sterile rubber policeman and washed before cell fusion.

Cell Fusion and Cloning. After enrichment for CId-1$^+$ cells, T cells were fused with the hypoxanthine guanosine phosphoribosyl transferase-resistant AKR (H-2^k) thymoma line BW 5147 and dispensed into 24-well culture dishes. Hybrid growth was detected 10–14 d after fusion. Screening for CId-1$^+$ T cell hybrids was performed using the indirect immunofluorescence assay described below. Hybrid cells were cloned by limiting dilution (22).

Immunofluorescence Screening for CId-1$^+$ Hybrids. The surface and cytoplasmic immunofluorescence techniques have been described (19). Capping of the CId-1$^+$ molecules was done by incubating CId-1 stained cells at 37°C for 20 min. Cell surface analysis was performed by fluorescence microscopy and on a fluorescence-activated cell sorter (FACS IV; B-D FACS Systems, Becton, Dickinson & Co.)

Lectin Treatment of CId-1$^+$ Hybrid Cells. CId-1B hybrid cells at 10^6/ml were cultured overnight at 37°C in the presence of 10–20 μg/ml of concanavalin A (Con A), lentil lectin (LL), wheat germ agglutinin (WGA) (Sigma Chemical Co., St. Louis, MO), pokeweed mitogen (PWM), and lipopolysaccharide (LPS), or with 2% phytohemagglutinin (PHA) (Gibco Laboratories). The surface distribution of the CId-1 determinant was then analyzed by immunofluorescence.

Treatment of CId-1$^+$ Hybrid Cells with Tunicamycin, Pronase, and Trypsin. CId-1B hybrid

cells at 2×10^6/ml were incubated with 0.5 μg/ml of tunicamycin (Calbiochem-Behring Corp., La Jolla, CA) in complete RPMI 1640 overnight at 37°C. For pronase and trypsin treatment, CId-1B cells were washed in Hanks' balanced salt solution (HBSS) at pH 7.2 and cultured at 2×10^6/ml with 50–100 μg/ml of pronase (Calbiochem-Behring Corp.) or 25–50 μg/ml of trypsin (Gibco Laboratories) in HBSS for 30 min at 37°C. The CId-1 expression was then analyzed by the FACS IV.

Results

The monoclonal CId-1 Antibody Cross-reacts with BALB/c Splenic T Lymphocytes. BALB/c spleen cell suspensions were stained by indirect immunofluorescence with the CId-1 anti-Id antibody, followed by rhodamine isothiocyanate (RITC)-conjugated goat antibodies to mouse μ chains, and counterstained with FITC-conjugated anti-Thy-1.2 or rat monoclonal anti-Lyt-1 or Lyt-2, followed by FITC-conjugated goat antibodies to rat IgG. Approximately 0.2% of Thy-1.2$^+$ BALB/c spleen cells co-stained with the monoclonal CId-1 antibody (Fig. 1). CId-1$^+$ BALB/c spleen cells were equally distributed between the Lyt-1$^+$ and the Lyt-2$^+$ T cell subsets. When the CId-1 antibody was replaced with a monoclonal anti-chicken Ia antibody (23) as a control IgM antibody in the staining procedure, no doubly stained cells were found.

Generation of CId-1$^+$ T Cell Hybrids. BALB/c splenic T cells, enriched for CId-1$^+$ cells (see Materials and Methods), were fused with the AKR BW 5147 cell line and dispensed into 216 wells. Among the resulting 72 wells with hybrid growth, two hybrids (CId-1A and CId-1B) reacted by indirect immunofluorescence with the monoclonal CId-1 anti-Id antibody and not with the CId-2 antibody. None of the remaining 70 T cell hybrids were reactive with either CId-1 or CId-2. Essentially all CId-1B hybrid cells stained with the CId-1 antibody in a ringlike pattern of discrete mini-patches on the cell surface (Fig. 2). The faint staining was confirmed by the FACS profiles of the CId-1A and CId-1B cells (Fig. 3). The fluorescence intensity exhibited by both the CId-1A and CId-1B hybrids was clearly above background, but ~10–20-fold less than that of BALB/c splenic B lymphocytes stained with goat anti-mouse μ-chain antibodies (data not shown). The CId-1 surface staining of the CId-1B hybrids was completely inhibited by preincubating the antibody with 20 μg of affinity-purified chicken anti-NAGA but not with 80 μg of anti-PABA antibodies.

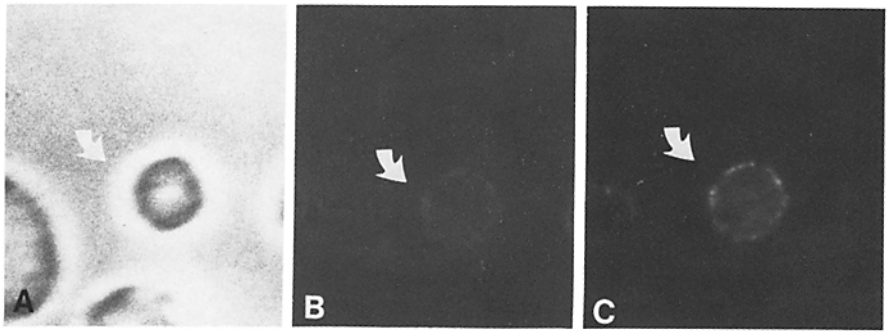

FIGURE 1. CId-1 expression by Thy-1.2$^+$ BALB/c spleen cells in a two-color immunofluorescence assay. The cell indicated by arrow co-expressed the CId-1 and Thy-1.2 determinants: (*A*) phase contrast, (*B*) anti-Thy-1.2 staining, and (*C*) CId-1 staining.

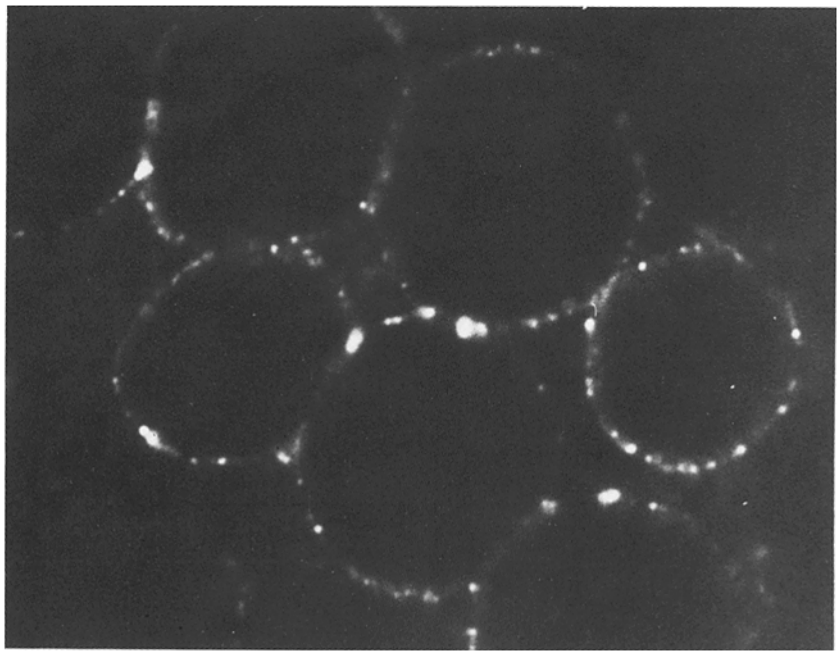

FIGURE 2. CId-1 surface immunofluorescence staining of the CId-1B hybrid cells illustrating the circumferential patchy staining pattern.

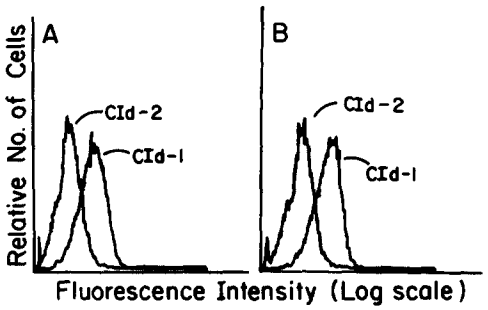

FIGURE 3. Fluorescence profiles of (*A*) CId-1A and (*B*) CId-1B hybrid cells stained by indirect immunofluorescence with CId-1 and CId-2 monoclonal antibodies.

To determine the intracellular distribution of CId-1 determinants, we examined fixed CId-1A and CId-1B cells. Diffuse patchy immunofluorescence with CId-1, but not with CId-2, antibody could be visualized in the cytoplasm of the CId-1A and B hybrid cells (Fig. 4).

Cell Surface Analysis of CId-1A and CId-1B Hybrids and the Parental AKR BW 5147 Line. The cell surface phenotypes were analyzed with a fluorescence microscope and the FACS (Table I). Both CId-1A and CId-B hybrid cells lacked CId-2 and mouse Ig heavy- and light-chain determinants (Fig. 5). They stained with the anti-H-2^d alloantiserum and with the monoclonal FITC-conjugated anti-Thy-1.2 antibody (Fig. 6*A*) and expressed the Lyt-1 antigen faintly (Fig. 6*B*) but lacked the Lyt-2 antigen. They expressed neither the parental I-A^d (BALB/c)

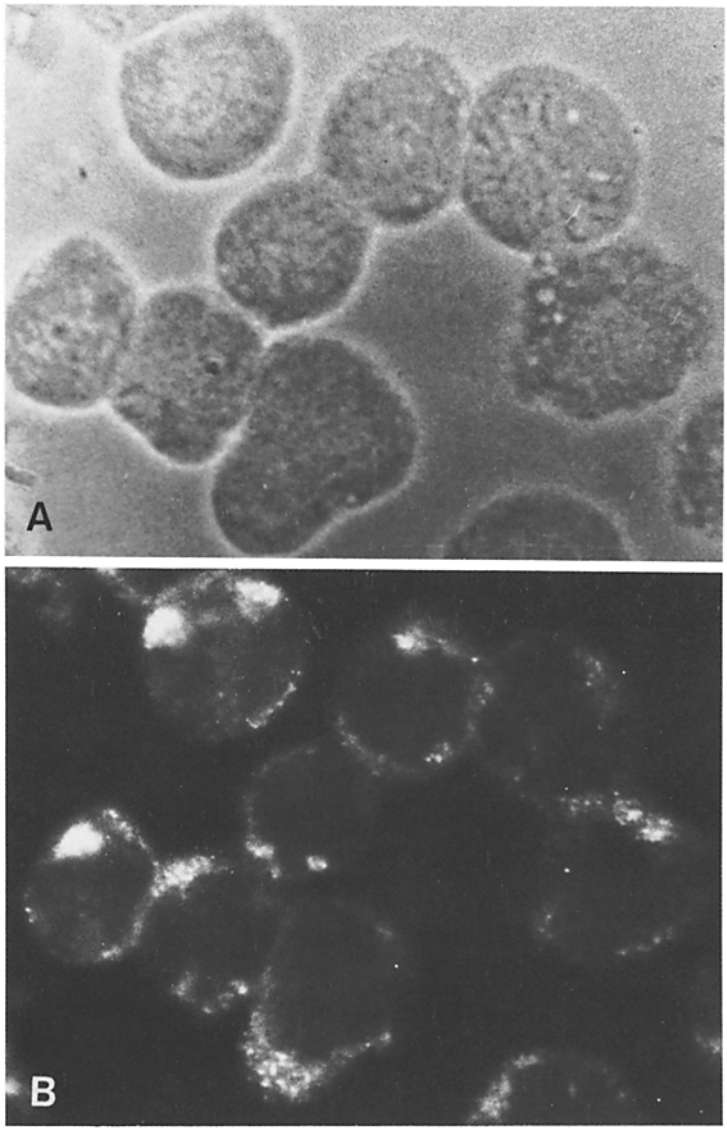

FIGURE 4. CId-1 cytoplasmic immunofluorescence staining of the CId-1B hybrid cells: (*A*) phase contrast and (*B*) CId-1 cytoplasmic staining. A similar staining pattern was observed with the CId-1A hybrid cells.

(Fig. 6*C*) nor the I-A^k (AKR) alleles but were positive for both the parental I-J^d and I-J^k alleles (Fig. 6*D*). The staining patterns of the CId-1B hybrids visualized by immunofluorescence with anti-Thy-1.2, anti-Lyt-1, anti-H-2^d, anti-I-J^d, and anti-I-J^k antibodies were all distinct from that seen with the CId-1 antibody.

The CId-1 Determinant Is Distinct from the Thy-1.2, Lyt-1, H-2^d, I-J^d, and I-J^k Molecules. To determine whether the CId-1 determinant was physically associated with Thy-1.2, Lyt-1, H-2^d, I-J^d, or I-J^k molecules on the cell membrane, CId-1B hybrid cells were incubated with the CId-1 antibody followed by RITC-

TABLE I

Immunofluorescence Analysis of Cell Surface Antigens on the CId-1A and CId-1B Hybrids and the Parental BW 5147 Cell Line

	Antibody specificities											
	CId-1	CId-2	Ig	κ and λ	H-2^d	Thy-1.2	Lyt-1	Lyt-2	I-A^d	I-A^k	I-J^d	I-J^k
BW5147	−	−	−	−	−	−	−	−	−	−	−	+
CId-1A	+	−	−	−	+	+	+	−	−	−	+	+
CId-1B	+	−	−	−	+	+	+	−	−	−	+	+

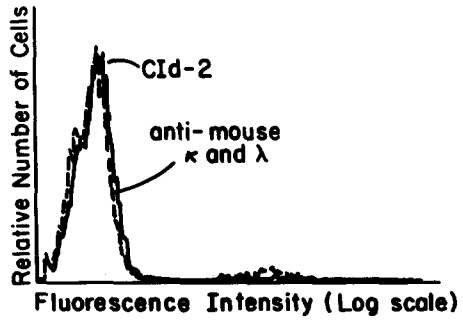

FIGURE 5. CId-1B hybrid cells lack light chain determinants. Fluorescence profiles of the CId-1B hybrid cells stained with CId-2 and affinity-purified goat anti-mouse κ- and λ-chain antibodies. Immunofluorescence reactivity of the hybrid cells was also not seen with antibodies to mouse Ig, μ, γ, δ, ε, and α determinants.

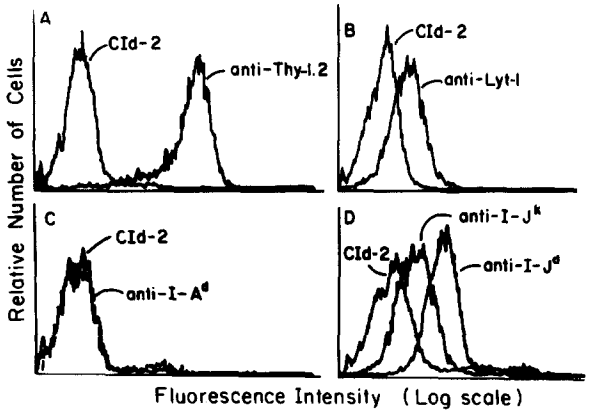

FIGURE 6. Fluorescence profiles of the CId-1B hybrid cells stained with (*A*) anti-Thy-1.2, (*B*) anti-Lyt-1, (*C*) anti-I-A^d, and (*D*) anti-I-J^d and anti-I-J^k antibodies.

conjugated goat antibodies to mouse μ-chains under capping conditions. The CId-1B cells were then stained either with CId-1 antibody followed by FITC-conjugated goat antibodies to mouse μ-chains to verify completeness of CId-1 capping, or with FITC-conjugated anti-Thy-1.2 antibody. After the CId-1 marker was capped (Fig. 7), Thy-1.2 molecules were still distributed over the entire surface of the hybrid cells. Similarly, CId-1 antibody-induced capping of the CId-1 determinant did not result in co-capping of Lyt-1, H-2^d, I-J^d, or I-J^k

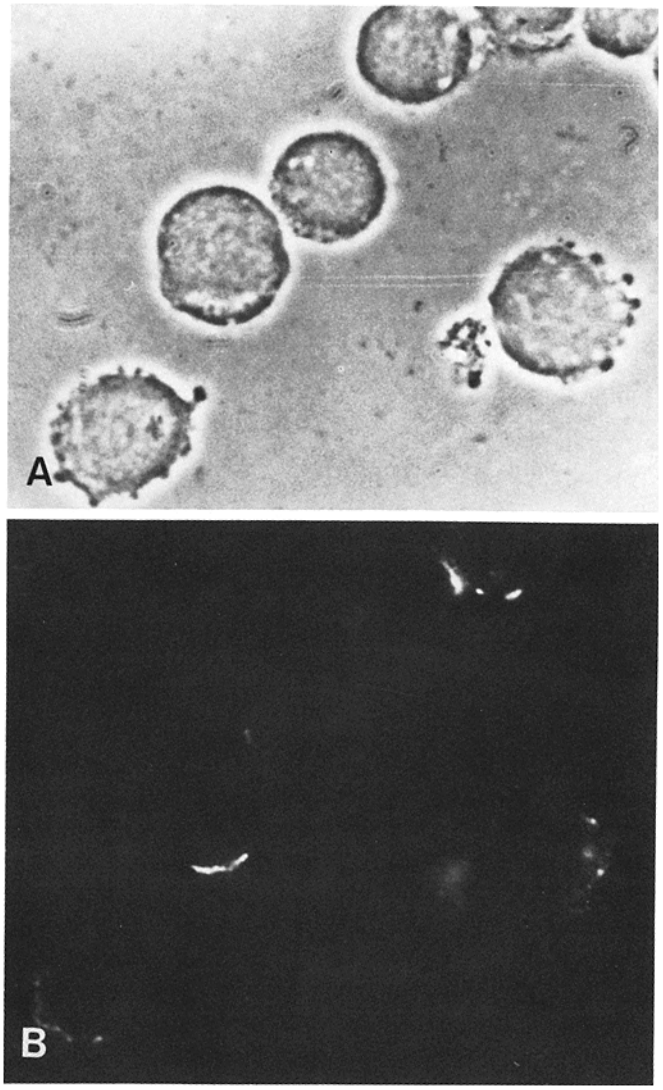

FIGURE 7. CId-1 antibody-induced capping of the CId-1 determinant does not result in redistribution of the Thy-1.2 antigen: (*A*) phase contrast, (*B*) CId-1 staining under capping conditions, and (*C*) anti-Thy-1.2 staining after capping of the CId-1 marker. Similar results were obtained with the H-2^d, Lyt-1, I-J^d, and I-J^k antigens.

molecules. Reverse capping experiments were then performed with anti-I-J^d and -I-J^k alloantibodies. Capping of the I-J^d and I-J^k molecules did not result in redistribution of the CId-1 determinant

Lectin-induced Modulation of the CId-1 Determinant. Since most cell surface proteins are glycoproteins, we tested a panel of lectins for their ability to bind to and modulate the CId-1 determinant on the CId-1B hybrid cells. Incubation of CId-1B hybrid cells with 10–20 μg/ml of Con A or LL at 37°C resulted in capping of the CId-1 marker to one cellular pole (Fig. 8). Incubation of CId-1B hybrids with PHA, PWM, LPS, or WGA had little or no apparent effect on the

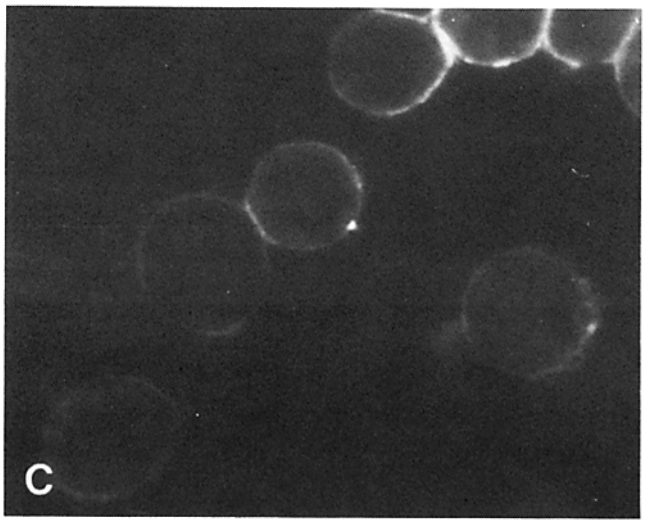

FIGURE 7C

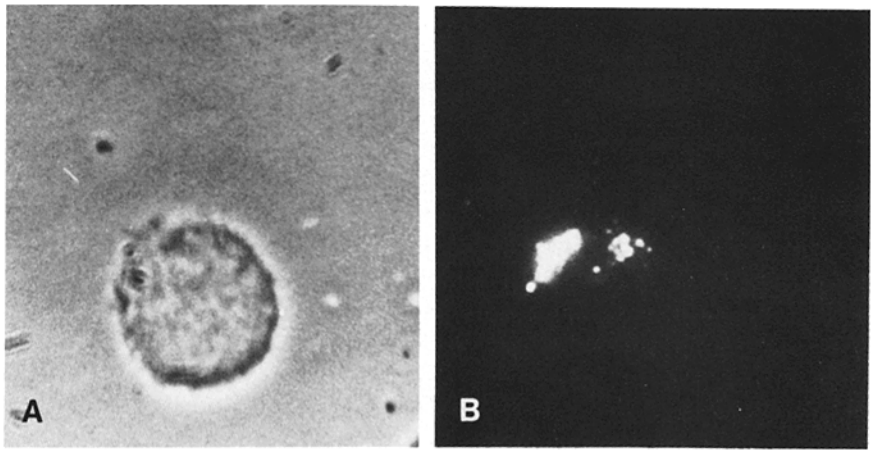

FIGURE 8. Con A-induced capping of the CId-1 determinant: (*A*) phase contrast, and (*B*) CId-1 staining after Con A treatment.

surface distribution of the CId-1 marker. Con A- and LL-induced capping of CId-1 determinants did not result in co-capping of Thy-1.2, Lyt-1, H-2^d, I-J^d, or I-J^k molecules. These results suggest that the CId-1 marker is a glycoprotein and further support the idea that the CId-1 determinant is distinct from the Thy-1.2, Lyt-1, H-2^d, I-J^d, and I-J^k antigens.

Effects of Tunicamycin, Pronase, and Trypsin on Expression of the CId-1 Determinant. Incubation of the CId-1B hybrids with 0.5 μg/ml of tunicamycin resulted in a shift of fluorescence intensity to near background level (Fig. 9*A*). In contrast, immunofluorescence analysis of fixed cells revealed that the cytoplasmic expression of CId-1 determinants was not reduced by the tunicamycin treatment. Incubation of CId-1B cells with 50–100 μg/ml of pronase or 25–50 μg/ml of trypsin also resulted in a shift of the fluorescence intensity of the treated cells to

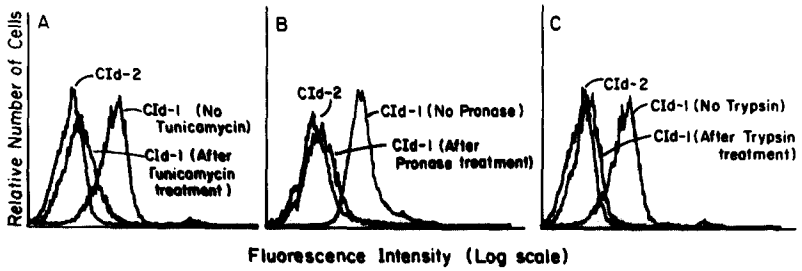

FIGURE 9. Fluorescence profiles of the CId-1B hybrid cells before and after treatment with (*A*) tunicamycin, (*B*) pronase, and (*C*) trypsin.

background level (Fig. 9, *B* and *C*). These results suggest that the CId-1 determinant is on a glycoprotein, and that glycosylation is required for normal surface expression of the molecule.

Discussion

The idiotope defined by the monoclonal CId-1 anti-Id antibody appeared to be a non–binding-site-associated idiotope on the heavy chain of chicken anti-NAGA antibodies, which suggests a V_H Id (19). The CId-1 Id was found to be conserved in all outbred and inbred chickens tested, as evidenced by its expression on ~20–25% of outbred and inbred chicken anti-NAGA antibodies and on ~1 and 0.4% of chicken B and T cells, respectively. Furthermore, the monoclonal CId-1 anti-Id antibody was cross-reactive with 0.2% of BALB/c mouse spleen cells that expressed the Thy-1.2 antigen. CId-1^+ BALB/c splenic T cells were found by indirect immunofluorescence within both the Lyt-1^+ and Lyt-2^+ T cell subsets. The fact that CId-1 is a mouse IgM antibody precluded testing of its reactivity with mouse B cells by indirect immunofluorescence, but ~0.5% of human plasma cells expressed CId-1^+ molecules (unpublished observation). Idiotypic cross-reactivity has been reported within inbred strains of mice (24, 25) and rabbits (26, 27), as well as between different strains of mice (28, 29). Idiotypic cross-reactivity has also been demonstrated between human and mouse, in the case of phosphorylcholine-binding myeloma proteins (30) and antibodies to acetylcholine receptor (31), and between goat and sheep antibodies to sickle cell hemoglobin (32).

Based on the observation that the CId-1 antibody reacted with a limited number of BALB/c mouse T cells, we fused enriched CId-1^+ BALB/c T cells with the AKR BW 5147 line and generated two of 72 hybrids, termed CId-1A and CId-1B, that reacted with the monoclonal CId-1 anti-Id antibody. Both cell hybrids lacked mouse Ig determinants and detectable Lyt-2 and I-A allelic determinants of both parental cells; each expressed the Thy-1.2, H-2^d, and I-J^d antigens of BALB/c origin, the I-J^k antigen of AKR origin and the Lyt-1 antigen. These results suggest that both CId-1A and CId-1B cells were T cell hybrids resulting from fusion events between BALB/c and AKR cells.

The relative immunofluorescence intensity of the CId-1 marker on CId-1A and CId-1B hybrids was ~10–20-fold less intense than that of BALB/c μ-bearing splenic B lymphocytes stained with the same preparation of goat antibodies to

mouse μ-chains. Assuming that a mature B lymphocyte expresses $\sim 10^5$ surface IgM molecules (33), the CId-1A and CId-1B hybrids would appear to express $\sim 5 \times 10^3$ CId-1$^+$ surface molecules per cell. This figure is consistent with the idea that the antigen-binding molecules on T cells may be 10–100-fold less dense than that expressed by B cells, and with the observation that T cell hybrids synthesize extremely small amounts of antigen-binding materials (34). A low density of antigen-binding molecules on the T cell surface could also contribute to the difficulty encountered in demonstrating specific antigen binding by T cells.

The binding of CId-1 antibody to the CId-1B hybrids was inhibited by preincubating the antibody with affinity-purified chicken antibodies to NAGA, but not by antibodies to PABA. The lack of binding to the T cell hybrids by the control CId-2 antibody and other mouse monoclonal antibodies of IgM isotype also strongly argues against the possibility of nonspecificity of the CId-1 binding to the CId-1B hybrid cells. Moreover, this possibility would not explain the specific immunofluorescent staining of cytoplasmic constituents in the CId-1A and -1B hybrid cells after fixation. The latter observation may also be pertinent to future biosynthetic studies of the CId-1 molecule.

The CId-1 surface marker could be easily capped by incubating CId-1B hybrid cells with the CId-1 monoclonal antibody at 37°C. CId-1 antibody-induced capping of the CId-1 determinant did not result in redistribution of other surface structures, including Thy-1.2, Lyt-1, H-2^d, I-J^d, and I-J^k. Similarly, capping of the I-J^d and I-J^k determinants did not alter the global distribution of the CId-1 determinant, which suggests that the CId-1 marker was not physically linked to these surface molecules.

Con A, in subagglutinating concentrations, has recently been reported to block the function of cytotoxic T cells, presumably by binding to surface structures essential for recognition or lysis of target cells (35). Incubation of CId-1B hybrids with Con A or LL resulted in the capping of the CId-1 determinant to one cellular pole, whereas incubation with PWM, PHA, LPS, or WGA did not alter surface distribution of the CId-1 determinant. The Con A-induced modulation of the CId-1 determinant did not result in a concomitant modulation of the Thy-1.2, Lyt-1, H-2^d, I-J^d, or I-J^k molecules, which further suggests that the CId-1 determinant is a distinctive cell surface component.

Treatment of CId-1B hybrids with tunicamycin, a compound that selectively prevents protein glycosylation (36), dramatically reduced surface expression of CId-1$^+$ molecules, but did not affect cytoplasmic expression of the antigen. Treatment of CId-1B hybrid cells with pronase or trypsin also resulted in a near-complete shift of fluorescence intensity of the CId-1 surface marker to background level. Taken together, these results suggest that the CId-1 antigen is on a protein molecule that is glycosylated en route to the cell surface, where it can be modulated by Con A or LL independently of the other surface structures recognized on the CId-1B hybrid cells.

It should be noted that we have no evidence of antigen binding or other functional activity for the CId-1$^+$ molecule on the T cell hybrids CId-1A and CId-1B. However, the pool size of circulating CId-1$^+$ T cells in the chicken was selectively increased after injections of either the CId-1 antibody or Strep A

organisms bearing the NAGA antigen (19). CId-1^+ T cells in mice represent a very small subpopulation (~0.2%) of the T cell pool. Clonal restriction in expression of this V_H idiotope is further emphasized by its low incidence (~3%) of expression by T cell hybrids that were produced by fusion of T cells, from an NAGA-immune donor, preselected by adherence to a CId-1 antibody-coated plate. We conclude that these T cell hybrids, which express a surface glycoprotein recognized by the monoclonal CId-1 antibody with V_H idiotope specificity, may provide a useful model system for identification and molecular characterization of the T cell antigen receptor.

Summary

Two mouse monoclonal antibodies to chicken immunoglobulin V_H-associated idiotypes (Id), CId-1 and CId-2, were used as probes for Id determinants on mouse T cells. CId-1, which recognized chicken antibodies to *N*-acetyl glucosamine (NAGA), and ~0.4% of chicken T lymphocytes also reacted with ~0.2% of BALB/c splenic Thy-1.2^+ cells. When enriched CId-1^+ splenic T cells from NAGA-immune BALB/c mice were fused with the AKR thymoma BW 5147 cell line, 2 of 72 resulting hybrids, termed CId-1A and CId-1B, were reactive by indirect immunofluorescence with the CId-1 antibody. CId-1 determinants were expressed both in the cytoplasm and on the cell surface. Immunofluorescence studies revealed that both CId-1^+ T cell hybrids were phenotypically identical: CId-2^-/Ig^-/Lyt-1^+2^-/Thy-1.2^+/H-2^{d+}/I-A^{d-}/I-A^{k-}/I-J^{d+}/I-J^{k+}. Incubation of CId-1B hybrid cells with concanavalin A or lentil lectin resulted in capping of the CId-1 determinant, whereas incubation with pokeweed mitogen, lipopolysaccharide, phytohemagglutinin, and wheat germ agglutinin had no effect on the cell surface distribution of the CId-1 molecule. Trypsin or pronase treatment resulted in the loss of detectable CId-1 determinant on the cell surface. Treatment of CId-1B cells with tunicamycin also reduced the immunofluorescence intensity of the surface CId-1 determinant, but had no effect on its cytoplasmic expression. CId-1 antibody-induced capping of the CId-1 marker did not affect the surface distribution of Lyt-1, Thy-1.2, H-2^d, I-J^d, or I-J^k molecules. Conversely, capping of I-J^d and I-J^k determinants did not alter the surface distribution of CId-1. These results suggest that the CId-1 determinant is on a glycoprotein that is not physically linked to the Lyt-1, Thy-1.2, H-2^d, I-J^d, and I-J^k molecules. The clonal restriction of CId-1 expression by T cells suggests that the CId-1^+ molecule could be a T cell antigen receptor.

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References

1. Warner, N. L., A. Szenberg, and F. M. Burnet. 1962. The immunological role of different lymphoid organs in the chicken. I. Dissociation of immunological respon-

siveness. *Aust. J. Exp. Biol. Med. Sci.* 40:373.
2. Cooper, M. D., R. D. A. Peterson, and R. A. Good. 1965. Delineation of the thymic and bursal lymphoid systems in the chicken. *Nature (Lond.).* 205:143.
3. Eichmann, K. 1975. Idiotype suppression. II. Amplification of a suppressor T cell with anti-idiotypic activity. *Eur. J. Immunol.* 5:511.
4. Eichmann, K., and K. Rajewsky. 1975. Induction of B and T cell immunity by anti-idiotypic antibodies. *Eur. J. Immunol.* 5:661.
5. Miller, G. G., P. J. Nadler, Y. Asano, R. J. Hodes, and D. H. Sachs. 1981. Induction of idiotype-bearing, nuclease-specific helper T cells by in vivo treatment with anti-idiotype. *J. Exp. Med.* 154:24.
6. Binz, H., and H. Wigzell. 1975. Shared idiotypic determinants on B and T lymphocytes reactive against the same antigenic determinants. I. Demonstration of similar or identical idiotypes on IgG molecules and T cell receptors with the same specificity for the same alloantigen. *J. Exp. Med.* 142:197.
7. Lewis, G. K., and J. W. Goodman. 1978. Purification of functional determinant-specific idiotype-bearing murine T cells. *J. Exp. Med.* 148:915.
8. Weinberger, J. F., R. N. Germain, S. T. Ju, M. I. Greene, B. Benacerraf, and M. E. Dorf. 1979. Hapten-specific T-cell responses to 4-hydroxy-3-nitrophenyl acetyl. II. Demonstration of idiotypic determinants on suppressor T cells. *J. Exp. Med.* 150:761.
9. Cerny, J., C. Heusser, R. Wallich, G. J. Hammerling, and D. D. Eardley. 1982. Immunoglobulin idiotypes expressed by T cells. I. Expression of distinct idiotypes detected by monoclonal antibodies on antigen-specific suppressor T cells. *J. Exp. Med.* 156:719.
10. Bach, B., M. I. Green, B. Benacerraf, and A. Nisonoff. 1979. Mechanisms of regulation of cell-mediated immunity. IV. Azobenzenearsonate-specific suppressor factor(s) bear cross-reactive idiotypic determinants the expression of which is linked to the heavy-chain allotype linkage group of genes. *J. Exp. Med.* 149:1084.
11. Kapp, J., B. A. Araneo, and B. L. Clevinger. 1980. Suppression of antibody and T cell proliferative responses to glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} by a specific monoclonal T cell factor. *J. Exp. Med.* 152:235.
12. Spurll, G. M., and F. L. Owen. 1981. A family of T-cell alloantigens linked to Igh-1. *Nature (Lond.).* 293:742.
13. Glassbrook, A. L., and F. W. Fitch. 1980. Alloreactive cloned T cell lines. I. Interactions between cloned amplifier and cytolytic T cell lines. *J. Exp. Med.* 151:876.
14. Fresno, M., L. McVay-Boudreau, G. Nabel, and H. Cantor. 1981. Antigen-specific T lymphocyte clones. II. Purification and biological characterization of an antigen-specific suppressive protein synthesized by cloned T cells. *J. Exp. Med.* 153:1260.
15. Meuer, S. C., K. A. Fitzgerald, R. E. Hussey, J. C. Hodgdon, S. F. Schlossmann, and E. L. Reinherz. 1983. Clonotypic structures involved in antigen specific human T cell function: relationship to the T_3 molecular complex. *J. Exp. Med.* 157:705.
16. Taniguchi, M., I. Takei, and T. Tada. 1980. Functional and molecular organization of an antigen-specific suppressor factor for a T-cell hybridoma. *Nature (Lond.).* 283:227.
17. Ruddle, N. H., B. Beezley, G. K. Lewis, and J. W. Goodman. 1980. Antigen-specific T cell hybrids. II. T cell hybrids which bind azobenzene-arsonate. *Mol. Immunol.* 17:925.
18. Sorensen, C. M., and C. W. Pierce. 1981. Characterization of a monoclonal haplotype-specific suppressor factor produced by a T-cell hybridoma. *In* Monoclonal Antibodies and T Cell Hybridomas. G. J. Hammerling, Y. Hammerling, and J. F. Kearney, editors. Elsevier/North-Holland, New York. 497
19. Chanh, T. C., C. L. Chen, and M. D. Cooper. 1982. Mouse monoclonal antibodies

to chicken V_H idiotypic determinants. Reactivity with B and T cells. *J. Immunol.* 129:2541.

20. Kearney, J. F., and A. R. Lawton. 1975. B lymphocyte differentiation induced by lipopolysaccharide. I. Generation of cells synthesizing four major immunoglobulin classes. *J. Immunol.* 115:671.
21. Wysocki, L. J., and V. L. Sato. 1978. "Panning" for lymphocytes: a method for cell selection. *J. Immunol.* 75:2844.
22. Hammerling, G. J., U. Hammerling, and J. K. Kearney. 1981. Production of antibody-producing hybridomas in the rodent systems. *In* Monoclonal Antibodies and T Cell Hybridomas. Elsevier/North-Holland, New York. 563.
23. Ewert, D. L., and M. D. Cooper. 1982. Ia-like antigens of the chicken. *In* Ia Antigens. II. Man and Other Species. S. Ferrone and C. S. David, editors. CRC Press, Inc., Boca Raton, FL. 1.
24. Potter, M., and R. Lieberman. 1970. Common individual antigenic determinants in five of eight BALB/c IgA myeloma proteins that bind phosphorylcholine. *J. Exp. Med.* 132:737.
25. Tung, A. S., and A. Nisonoff. 1975. Isolation from individual A/J mice of anti-*p*-azophenylarsonate antibodies bearing a cross-reactive idiotype. *J. Exp. Med.* 141:112.
26. Eichmann, K., and T. Kindt. 1971. The inheritance of individual antigenic specificities of rabbit antibodies to streptococcal carbohydrates. *J. Exp. Med.* 134:532.
27. Kindt, T. S., R. K. Seide, V. A. Bockisch, and R. M. Krause. 1973. Detection of idiotypic cross-reactions among streptococcal antisera from related rabbits. *J. Exp. Med.* 138:522.
28. Briles, D., and R. M. Krause. 1974. Mouse strain-specific idiotypy and interstrain idiotypic cross-reactions. *J. Immunol.* 113:522.
29. Claflin, J. L., and J. M. Davies. 1975. Clonal nature of the immune response to phosphorylcholine (PC). V. Cross-idiotypic specificity among heavy chains of murine anti-PC antibodies and PC-binding myeloma proteins. *J. Exp. Med.* 141:1073.
30. Riesen, W. F., D. G. Braun, and J. C. Jaton. 1976. Human and mouse phosphorylcholine-binding immunoglobulins: conserved subgroup and first hypervariable region of heavy chains. *Proc. Natl. Acad. Sci. USA.* 73:2096.
31. Dwyer, D. S., R. J. Bradley, C. K. Urquhart, and J. F. Kearney. 1983. Naturally occurring anti-idiotypic antibodies in myasthenia gravis. *Nature* (*Lond.*). 301:611.
32. Karol, R. A., M. Reichlin, and R. W. Noble. 1977. Evolution of an idiotypic determinant: anti-val. *J. Exp. Med.* 145:435.
33. Rabellino, E., S. Colon, H. M. Grey, and E. R. Unanue. 1971. Immunoglobulins on the surface of lymphocytes. I. Distribution and quantitation. *J. Exp. Med.* 133:156.
34. James, R. F. L., S. Kontiainen, D. J. Maudsley, E. J. Culbert, and M. Feldmann. 1983. A monoclonal antibody against antigen-specific helper factor augments T-cell help. *Nature* (*Lond.*). 301:160.
35. Sitkovsky, M. J., M. S. Paternack, and H. N. Eisen. 1982. Inhibition of cytotoxic T lymphocyte activity by concanavalin A. *J. Immunol.* 129:1372.
36. Tkacz, J. S., and J. O. Lampen. 1975. Tunicamycin inhibition of polyisoprenyl-*N*-acetylglucosaminyl pyrophosphate formation in calf-liver microsomes. *Biochem. Biophys. Res. Commun.* 65:248.