T cell hybrids that express a VH idiotope-related determinant on a glycoprotein distinct from H-2, Thy-1, and Lyt-1 molecules

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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)\textsuperscript{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\textsubscript{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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\textsuperscript{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; IL, 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.
exclusively with Ig expressed by B cells. We have since found that the Cld-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 Cld-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 Cld-1 monoclonal antibody. In this paper, we describe the generation of these T cell hybrids and an initial characterization of their Cld-1 determinants.

Materials and Methods

Antisera. The preparation and characterization of the monoclonal Cld-1 and Cld-2 anti-Id antibodies (IgMx) have been described (19). The rat monoclonal antibodies to mouse Lyt-1 and Lyt-2, the mouse monoclonal antibodies to mouse I-A<sup>d</sup> and I-A<sup>A</sup> and monoclonal fluorescein isothiocyanate (FITC)-conjugated anti-Thy-1.2 antibodies were from Becton, Dickinson & Co., Sunnyvale, CA. The mouse anti-I-J<sup>d</sup> and I-J<sup>k</sup> alloantisera were gifts from Dr. Chella S. David, Mayo Clinic, Rochester, MN. The anti-H-2<sup>d</sup> 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<sup>d</sup>) were immunized intraperitoneally three times at 5-d intervals with 10<sup>9</sup> heat-killed Streptococcus group A strain J17A4 (Strep A) organisms.

Enrichment of Cld-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 Cld-1+ T lymphocytes, the nonadherent cells were treated with 200 µg/ml of the Cld-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<sup>-5</sup> 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 Cld-1+ cells, T cells were fused with the hypoxanthine guanosine phosphoribosyl transferase-resistant AKR (H-2<sup>k</sup>) thymoma line BW 5147 and dispensed into 24-well culture dishes. Hybrid growth was detected 10-14 d after fusion. Screening for Cld-1+ T cell hybrids was performed using the indirect immunofluorescence assay described below. Hybrid cells were cloned by limiting dilution (22).

Immunofluorescence Screening for Cld-1+ Hybrids. The surface and cytoplasmic immunofluorescence techniques have been described (19). Capping of the Cld-1 molecules was done by incubating Cld-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 Cld-1+ Hybrid Cells. Cld-1B hybrid cells at 10<sup>6</sup>/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 Cld-1 determinant was then analyzed by immunofluorescence.

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

**Results**

*The monoclonal Cld-1 Antibody Cross-reacts with BALB/c Splenic T Lymphocytes.* BALB/c spleen cell suspensions were stained by indirect immunofluorescence with the Cld-1 anti-Id antibody, followed by rhodamine isothiocyanate (RITC)-conjugated goat antibodies to mouse $\mu$ 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 Cld-1 antibody (Fig. 1). Cld-1+ BALB/c spleen cells were equally distributed between the Lyt-1+ and the Lyt-2+ T cell subsets. When the Cld-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 Cld-1+ T Cell Hybrids.* BALB/c splenic T cells, enriched for Cld-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 (Cld-1A and Cld-1B) reacted by indirect immunofluorescence with the monoclonal Cld-1 anti-Id antibody and not with the Cld-2 antibody. None of the remaining 70 T cell hybrids were reactive with either Cld-1 or Cld-2. Essentially all Cld-1B hybrid cells stained with the Cld-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 Cld-1A and Cld-1B cells (Fig. 3). The fluorescence intensity exhibited by both the Cld-1A and Cld-1B hybrids was clearly above background, but ~10–20-fold less than that of BALB/c splenic B lymphocytes stained with goat anti-mouse $\mu$-chain antibodies (data not shown). The Cld-1 surface staining of the Cld-1B hybrids was completely inhibited by preincubating the antibody with 20 $\mu$g of affinity-purified chicken anti-NAGA but not with 80 $\mu$g of anti-PABA antibodies.
To determine the intracellular distribution of Cld-1 determinants, we examined fixed Cld-1A and Cld-1B cells. Diffuse patchy immunofluorescence with Cld-1, but not with Cld-2, antibody could be visualized in the cytoplasm of the Cld-1A and B hybrid cells (Fig. 4).

Cell Surface Analysis of Cld-1A and Cld-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 Cld-1A and Cld-B hybrid cells lacked Cld-2 and mouse Ig heavy- and light-chain determinants (Fig. 5). They stained with the anti-H-2d alloantiserum and with the monoclonal FITC-conjugated anti-Thy-1.2 antibody (Fig. 6A) and expressed the Lyt-1 antigen faintly (Fig. 6B) but lacked the Lyt-2 antigen. They expressed neither the parental I-A^d (BALB/c)
(Fig. 6C) nor the I-A^k (AKR) alleles but were positive for both the parental I-J^d and I-J^k alleles (Fig. 6D). The staining patterns of the Cld-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 Cld-1 antibody.

The Cld-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 Cld-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, Cld-1B hybrid cells were incubated with the Cld-1 antibody followed by RITC-
TABLE I

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

| Antibody specificities | Cld-1 | Cld-2 | Ig⁻ and λ | H-2k | Thy-1.2 | Lyt-1 | Lyt-2 | I-Ak | I-Ak | I-Jk | I-Jk |
|------------------------|-------|-------|-----------|------|---------|-------|-------|------|------|------|------|
| BW5147                 | -     | -     | -         | -    | -       | +     | +     | -    | -    | +    | +    |
| Cld-1A                 | +     | -     | -         | -    | +       | -     | -     | -    | -    | +    | +    |
| Cld-1B                 | +     | -     | -         | -    | +       | -     | -     | -    | -    | +    | +    |

**Figure 5.** Cld-1B hybrid cells lack light chain determinants. Fluorescence profiles of the Cld-1B hybrid cells stained with Cld-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 Cld-1B hybrid cells stained with (A) anti-Thy-1.2, (B) anti-Lyt-1, (C) anti-I-Ak, and (D) anti-I-Jk and anti-I-Jk antibodies.

Conjugated goat antibodies to mouse μ-chains under capping conditions. The Cld-1B cells were then stained either with Cld-1 antibody followed by FITC-conjugated goat antibodies to mouse μ-chains to verify completeness of Cld-1 capping, or with FITC-conjugated anti-Thy-1.2 antibody. After the Cld-1 marker was capped (Fig. 7), Thy-1.2 molecules were still distributed over the entire surface of the hybrid cells. Similarly, Cld-1 antibody-induced capping of the Cld-1 determinant did not result in co-capping of Lyt-1, H-2k, I-Jk, or I-Jk.
Figure 7. Clq-1 antibody-induced capping of the Clq-1 determinant does not result in redistribution of the Thy-1.2 antigen: (A) phase contrast, (B) Clq-1 staining under capping conditions, and (C) anti-Thy-1.2 staining after capping of the Clq-1 marker. Similar results were obtained with the H-2S, Lyt-1, I-Jα, and I-Jβ antigens.

molecules. Reverse capping experiments were then performed with anti-I-Jα and -I-Jβ alloantibodies. Capping of the I-Jα and I-Jβ molecules did not result in redistribution of the Clq-1 determinant.

Lectin-induced Modulation of the Clq-1 Determinant. Since most cell surface proteins are glycoproteins, we tested a panel of lectins for their ability to bind to and modulate the Clq-1 determinant on the Clq-1B hybrid cells. Incubation of Clq-1B hybrid cells with 10-20 µg/ml of Con A or LL at 37°C resulted in capping of the Clq-1 marker to one cellular pole (Fig. 8). Incubation of Clq-1B hybrids with PHA, PWM, LPS, or WGA had little or no apparent effect on the
surface distribution of the Cld-1 marker. Con A- and LL-induced capping of Cld-1 determinants did not result in co-capping of Thy-1.2, Lyt-1, H-2d, I-Jd, or I-Jk molecules. These results suggest that the Cld-1 marker is a glycoprotein and further support the idea that the Cld-1 determinant is distinct from the Thy-1.2, Lyt-1, H-2d, I-Jd, and I-Jk antigens.

**Effects of Tunicamycin, Pronase, and Trypsin on Expression of the Cld-1 Determinant.** Incubation of the Cld-1B hybrids with 0.5 μg/ml of tunicamycin resulted in a shift of fluorescence intensity to near background level (Fig. 9A). In contrast, immunofluorescence analysis of fixed cells revealed that the cytoplasmic expression of Cld-1 determinants was not reduced by the tunicamycin treatment. Incubation of Cld-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
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Figure 9. Fluorescence profiles of the Cld-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 Cld-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 Cld-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 VH Id (19). The Cld-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 Cld-1 anti-Id antibody was cross-reactive with 0.2% of BALB/c mouse spleen cells that expressed the Thy-1.2 antigen. Cld-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 Cld-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 Cld-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 Cld-1 antibody reacted with a limited number of BALB/c mouse T cells, we fused enriched Cld-1+ BALB/c T cells with the AKR BW 5147 line and generated two of 72 hybrids, termed Cld-1A and Cld-1B, that reacted with the monoclonal Cld-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-2d, and I-Jd antigens of BALB/c origin, the I-Jk antigen of AKR origin and the Lyt-1 antigen. These results suggest that both Cld-1A and Cld-1B cells were T cell hybrids resulting from fusion events between BALB/c and AKR cells.

The relative immunofluorescence intensity of the Cld-1 marker on Cld-1A and Cld-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 $\mu$-chains. Assuming that a mature B lymphocyte expresses $\sim10^5$ surface IgM molecules (33), the Cld-1A and Cld-1B hybrids would appear to express $\sim5 \times 10^5$ Cld-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 Cld-1 antibody to the Cld-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 Cld-2 antibody and other mouse monoclonal antibodies of IgM isotype also strongly argues against the possibility of nonspecificity of the Cld-1 binding to the Cld-1B hybrid cells. Moreover, this possibility would not explain the specific immunofluorescent staining of cytoplasmic constituents in the Cld-1A and -1B hybrid cells after fixation. The latter observation may also be pertinent to future biosynthetic studies of the Cld-1 molecule.

The Cld-1 surface marker could be easily capped by incubating Cld-1B hybrid cells with the Cld-1 monoclonal antibody at 37°C. Cld-1 antibody-induced capping of the Cld-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 Cld-1 determinant, which suggests that the Cld-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 Cld-1B hybrids with Con A or LL resulted in the capping of the Cld-1 determinant to one cellular pole, whereas incubation with PWM, PHA, LPS, or WGA did not alter surface distribution of the Cld-1 determinant. The Con A-induced modulation of the Cld-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 Cld-1 determinant is a distinctive cell surface component.

Treatment of Cld-1B hybrids with tunicamycin, a compound that selectively prevents protein glycosylation (36), dramatically reduced surface expression of Cld-1$^+$ molecules, but did not affect cytoplasmic expression of the antigen. Treatment of Cld-1B hybrid cells with pronase or trypsin also resulted in a near-complete shift of fluorescence intensity of the Cld-1 surface marker to background level. Taken together, these results suggest that the Cld-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 Cld-1B hybrid cells.

It should be noted that we have no evidence of antigen binding or other functional activity for the Cld-1$^+$ molecule on the T cell hybrids Cld-1A and Cld-1B. However, the pool size of circulating Cld-1$^+$ T cells in the chicken was selectively increased after injection of either the Cld-1 antibody or Strep A.
organisms bearing the NAGA antigen (19). Cld-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 Cld-1 antibody-coated plate. We conclude that these T cell hybrids, which express a surface glycoprotein recognized by the monoclonal Cld-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), Cld-1 and Cld-2, were used as probes for Id determinants on mouse T cells. Cld-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 Cld-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 Cld-1A and Cld-1B, were reactive by indirect immunofluorescence with the Cld-1 antibody. Cld-1 determinants were expressed both in the cytoplasm and on the cell surface. Immunofluorescence studies revealed that both Cld-1 + T cell hybrids were phenotypically identical: Cld-2-/Ig-/Lyt-1.2-/Thy-1.2+/H-2d+/I-A^d+/I-A^k-/I-J^d+/I-J^k+. Incubation of Cld-1B hybrids with concanavalin A or lentil lectin resulted in capping of the Cld-1 determinant, whereas incubation with pokeweed mitogen, lipopolysaccharide, phytohemagglutinin, and wheat germ agglutinin had no effect on the cell surface distribution of the Cld-1 molecule. Trypsin or pronase treatment resulted in the loss of detectable Cld-1 determinant on the cell surface. Treatment of Cld-1B cells with tunicamycin also reduced the immunofluorescence intensity of the surface Cld-1 determinant, but had no effect on its cytoplasmic expression. Cld-1 antibody-induced capping of the Cld-1 marker did not affect the surface distribution of Lyt-1, Thy-1.2, H-2d, 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 Cld-1. These results suggest that the Cld-1 determinant is on a glycoprotein that is not physically linked to the Lyt-1, Thy-1.2, H-2d, I-J^d, and I-J^k molecules. The clonal restriction of Cld-1 expression by T cells suggests that the Cld-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-
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-idiotype 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 determinantspecific 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. Azobenzene arsonate-specific suppressor factor(s) bear cross-reactive idiotypic determinants the expression of which is linked to the heavy-chain allele linkage group of genes. *J. Exp. Med.* 149:1084.

11. Kapp, J., B. A. Araneo, and B. L. Cleveinger. 1980. Suppression of antibody and T cell proliferative responses to glutamic acid<sup>69</sup>-L-alanine<sup>50</sup>-L-tyrosine<sup>10</sup> 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<sub>3</sub> 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. Beersley, 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 haplootype-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 *La 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 idiotyp 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.