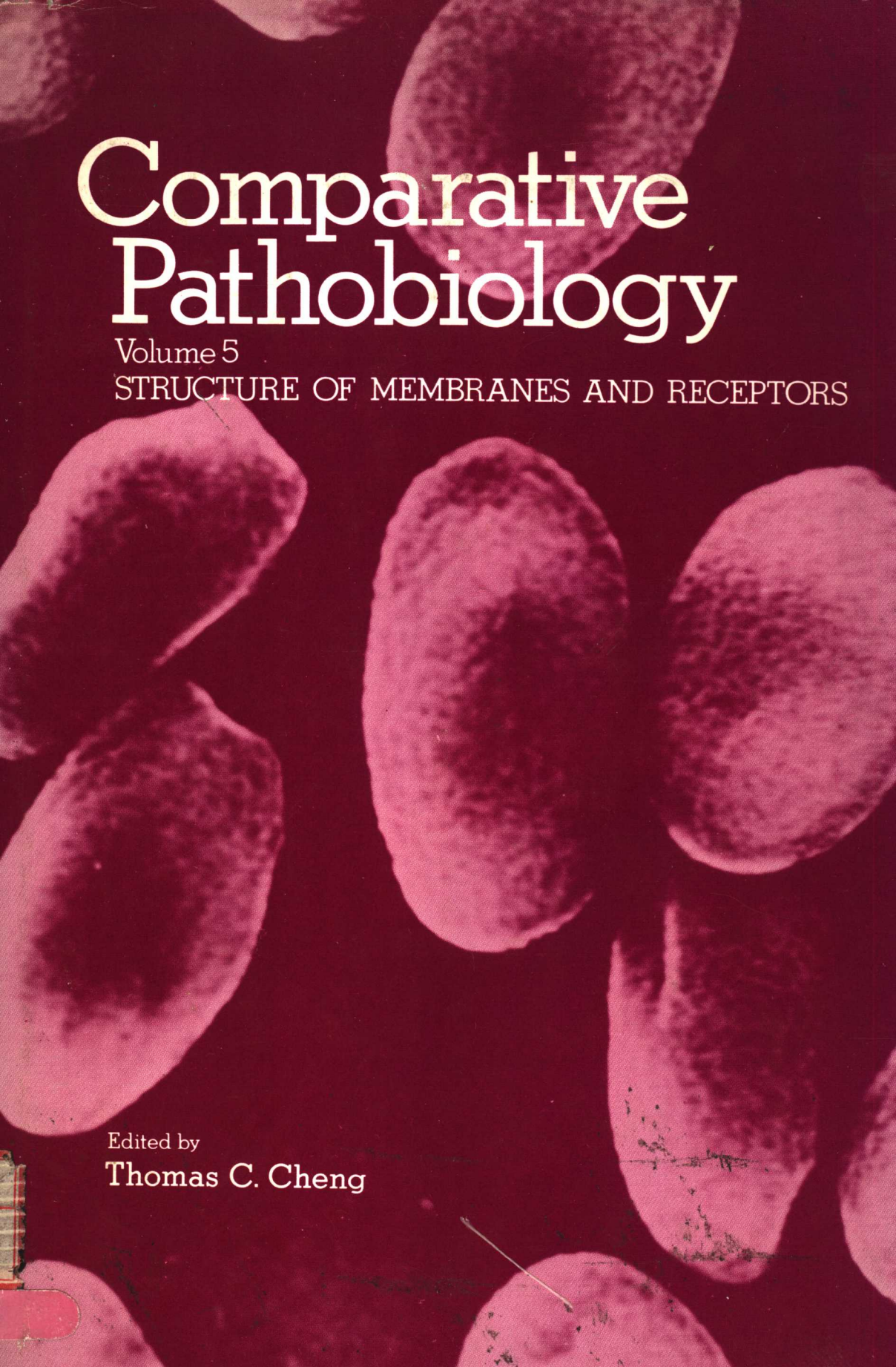
Comparative Pathobiology

Volume 5

STRUCTURE OF MEMBRANES AND RECEPTORS



Edited by

Thomas C. Cheng

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Volume 5

STRUCTURE OF MEMBRANES AND RECEPTORS

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Medical University of South Carolina
Charleston, South Carolina

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Proceedings of a special symposium on Structure of Membranes and Receptors, held February 19–20, 1981, at the Medical University of South Carolina, Charleston, South Carolina

PREFACE

On February 19 and 20, 1981, a special symposium was held at the Medical University of South Carolina in Charleston. The common theme of this series of presentations was "Structure of Membranes and Receptors." Although most of the papers were by members of the faculty of this institution, through a grant from the office of Dr. John W. Zemp, Dean of the College of Graduate Studies, we were fortunate to have had with us Dr. Lee Hood of the California Institute of Technology, Dr. Michael J. Crumpton of the Imperial Cancer Research Fund, United Kingdom, and Dr. David D. Sabatini of the New York University Medical Center.

This symposium, for which the following pages represent the proceedings, was organized by Dr. John J. Marchalonis of the Department of Biochemistry and Dr. Edward L. Hogan of the Department of Neurology, both of the Medical University of South Carolina.

The common theme is a timely one since, as any reader will recognize, the structure of membranes and receptors, whether pertaining to cellular immunology, developmental biology, microbiology, cellular endocrinology, or experimental neurology, represents a major cutting edge of modern biology today. The organization and participants of the symposium enthusiastically endorsed the idea that what was presented in Charleston, South Carolina, should be shared with colleagues around the world. Consequently, plans were made to publish these proceedings as Volume 5 of Comparative Pathobiology.

The editor wishes to take this opportunity to announce that Dr. Lee A. Bulla, Jr. the former co-editor of Comparative Pathobiology, has elected to retire from this responsibility, leaving me, at least for the time being, as the sole editor. The original idea that stimulated the launching of this series by Plenum Press, New York and London, however, remains unaltered.

PREFACE

Comparative Pathobiology is intended for the publication of the proceedings of special symposia dealing with all aspects of what is commonly designated as pathobiology that the editor deems worthwhile sharing with the scientific community. Those interested in supporting this objective should contact the editor.

Thomas C. Cheng
Charleston, South Carolina

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IMMUNOGLOBULIN-RELATED ANTIGEN-RECEPTORS OF THYMUS-DERIVED LYMPHOCYTES[1]

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I. INTRODUCTION

Lymphocytes are small round cells of rather unprepossessing appearance, yet they possess a potential for recognition of foreignness and for differentiation into effector cells that makes them a prominent bulwark of the body's defenses. Two broad functional classes of lymphocytes have been described and studied with great vigor during the past 15 years. These are the B cells, or bone-marrow-derived lymphocytes, which are the precursors of antibody forming cells, and the so-called T cells for thymus-derived lymphocytes. T cells play the major role in cell mediated immunity, e.g., the rejection of tumors and allografts, and are required for collaboration with B cells in the elaboration of immune responses

1. Supported in part by grant RD-101 from the American Cancer Society and 1R01/AI17493-01 from the National Institutes of Health.

to many antigens (Marchalonis, 1977a). The mechanisms by which T cells interact with one another, with nonlymphoid accessory cells and with B cells are extremely complicated and various models have been proposed for the regulation of an immunological network. I will not consider the mechanisms here but will focus upon the molecular properties of the primary receptor for antigen as it occurs on the surface of antigen-specific T cells. It is clear at this time that in both man and mouse the antigen receptor on B cells resembles serum immunoglobulin and bears not only immunoglobulin combining site regions but class specific determinants of the IgM and IgD isotypes (Vitetta and Uhr, 1975). The problem of identifying and characterizing the antigen-specific receptor on T cells has been a controversial and challenging issue in cellular and molecular immunology since the days when it was first recognized that T cells could interact specifically with antigen. The minimal hypothesis regarding this receptor is that the T cell surface should possess combining site similar or identical to those immunoglobulin variable (V) regions but its constant regions are not necessarily identical to those of circulating antibodies. This hypothesis is illustrated in Fig. 1 which compares a B cell IgM receptor with circulating IgG antibody to the same antigen. In order to test this hypothesis, it was necessary to derive a battery of antisera directed against combining site region determinants of serum antibodies and to develop means for detecting whether such antibodies reacted with T cells and with T cell products. In addition, it was necessary to develop a sensitive radiochemical method for labeling external surface proteins of plasma membranes and subsequently using the reactive antibodies as probes for the isolation of the surface molecules (Marchalonis et al., 1971). I will present data supporting the conclusion that certain, antigen-specific T cells express and produce molecules related, but not identical, to serum immunoglobulins and will describe some biochemical characterization data comparing the T cell immunoglobulin-like receptor with classical serum antibodies.

II. EXPERIMENTAL EVIDENCE

We have used two approaches to the problem of plasma membrane receptors of T and B cells. One approach was to use the enzyme lactoperoxidase as a catalyst to incorporate carrier-free ^{125}I-iodide into tyrosyl residues of exposed protein on the external face of the plasma membrane (Marchalonis et al., 1971). This approach provided sufficient sensitivity to enable us to carry out microanalytical studies on the serological and biochemical properties of receptors isolated from a few as 2×10^6 T cells. The other approach which we used was based upon the fractionation procedures for membranes devised by Crumpton and Snary (1974) which entailed disruption of the cells, starting with approximately 10^{10} or more lymphocytes, and isolation of the plasma membrane by differential

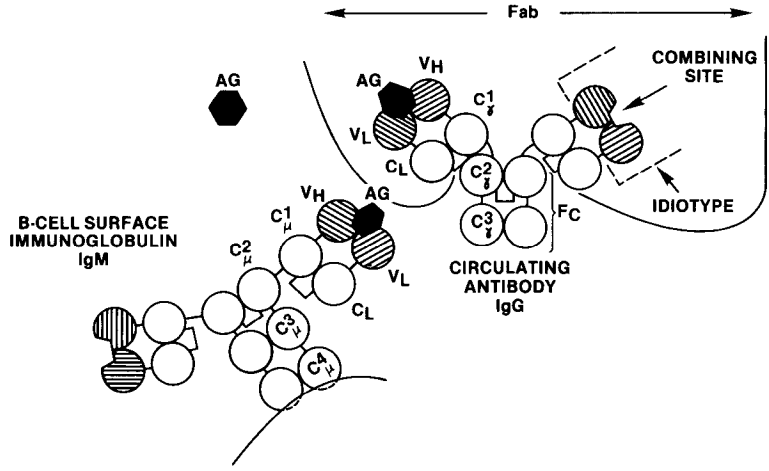

Fig. 1. Diagram illustrating the similarities and differences between B cell surface IgM receptors for a given antigen and the circulating IgG antibody to that antigen. Ag antigen; Fab, antigen binding fragment; Fc, Fc portion of IgG immunoglobulin comprised of the second and third constant region domains. The Fc fragment of the surface μ chain would be comprised of constant region domains of the μ chain. Although the circulating antibody and the surface IgM have the same combining site for antigen they represent different classes as defined by their heavy chains. Furthermore, surface μ chain has a hydrophobic tail which allows it to intercalate with the plasma membrane. It differs in this structure from the μ chain of circulating IgM. The IgM plasma membrane molecule is also a 7S structure composed of two Lμ pairs, rather than the cyclic pentamer characteristic of secreted antibody. Idiotypic determinants are combining site region-related determinants and are usually formed by interaction between the V_H and V_L portions of the molecules, although some idiotypic determinants are specified predominantly by the V_H structure.

centrifugation. As can be seen in Fig. 2 which presents a stained pattern of the plasma membrane proteins of the T cell lymphoma WEHI 22 as resolved on polyacrylamide gel electrophoresis in sodium dodecyl sulfate, more than 50 bands can be detected. Because of the complexity of the patterns, it was necessary to use affinity techniques to isolate particular components. We have used three types of approaches: In the first approach we used affinity chromatography on insolubilized lectins in order to isolate glycosylated membrane proteins bearing terminal sugars of particular sequences (Hunt and Marchalonis, 1974); the second approach was to use insolubilized antibodies as our immune affinity reagent (Hunt and Marchalonis, 1974); and in the third approach, we isolated ligand-binding surface proteins using insolubilized ligand such as the hapten dinitrophenyl coupled to Sepharose (Marchalonis, 1976).

The great selective power of immune affinity chromatography is illustrated in Fig. 3 which presents an SDS-polyacrylamide gel of radioiodinated surface immunoglobulins of murine B cells which were isolated in a single step by binding to rabbit antibody to mouse κ light chain coupled to Sepharose 4B. Only three components can be detected in this autoradiograph. One is the μ chain of the cell surface 7S IgM; another is the δ chain of cell surface IgD which can appear on the same cell; and the third component is the light chain (κ), which can occur in covalent association with either the μ or δ chain. The δ chain envelope is broad relative to that of μ chain, and this is because the δ chain from the cell surface shows considerable heterogeneity, possibly due either to differing degrees of glycosylation or to proteolysis.

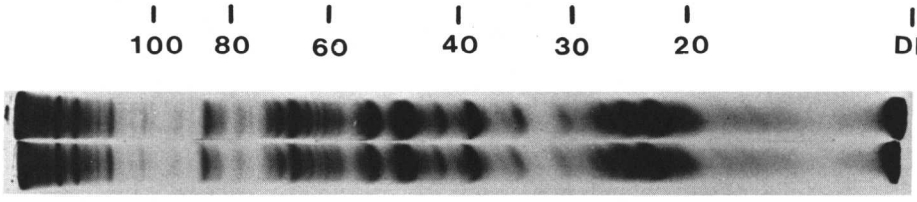

Fig. 2. Resolution by polyacrylamide gel electrophoresis in sodium dodecyl-containing buffers in the presence of mercaptoethanol of isolated plasma membranes of the murine T cell lymphoma WEHI 22. DM, dyemaker. The numbers indicate the position at which proteins of known molecular weight migrate under these gel conditions (MW x 1000).

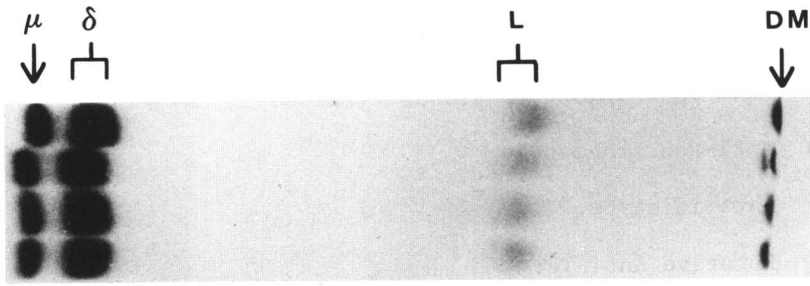

Fig. 3. Radioautograph of ^{125}I-labeled surface immunoglobulins of murine B cells which were surface-radioiodinated using the lactoperoxidase catalyzed technique (Marchalonis et al., 1971) and isolated as a single step by immune affinity chromatography using insolubilized antibody to mouse κ chain. Four replicate samples were resolved by polyacrylamide gel electrophoresis in sodium dodecylsulfate-containing buffers in the presence of mercaptoethanol. DM, dyemaker. L indicates the position at which light chains migrate; μ and δ refer to the B cell surface μ and δ chains. B cell 7S IgM exists in the form $(\kappa\mu)_2$; cell surface IgD exists in the form $(\kappa\delta)_2$.

In studies of the T cell receptor, we and others found that antisera which were specific for the constant regions of the heavy chains or light chains, i.e., the antibodies which reacted with the dominant isotype-specific determinants, were not reactive with the T cell product. However, it was found that certain antiserum made against normal, polyclonal serum immunoglobulins did react with T cell products (Marchalonis et al., 1972; Burckhardt et al., 1974), and, moreover, antibodies made against individually specific determinants (idiotypes) of antibodies or homogenous myeloma proteins did react with T cell products (Binz and Wigzell, 1977; Rajewsky and Eichmann, 1977).

Table 1 lists the type of antibodies which in our hands were found to react with T cell receptors for antigen. In essence, the first four types of antibodies are ones which have been raised against the combining site region either of polyclonal, normal immunoglobulins in the case of the chicken antibodies against Fab interaction determinant or reactive with combining site determinants of particular antibodies. See Fig. 1 above for graphic representation of the location of these fragments. In our case, we raised rabbit antibodies against the combining site of murine antibodies

Table 1. Antibodies which react with T cell receptors for antigen.

(1) Anti-fab interaction determinants (raised in chickens)

(2) Anti-V_H fragment of μ chain (raised in chickens)

(3) Anti-Fdμ fragment

(4) Anti-idiotype

(5) Putative Anti-TgT

from strain A/J mice which reacted with the arsonate hapten (Tung and Nisonoff, 1975; Marchalonis et al., 1979). Antibodies of type 1 which are directed against a polyclonal pool of Fab interaction determinants can be termed "shotguns" because they react with the vast majority of the pool of Fab interaction determinants as it occurs in serum and on cells. By contrast, the so-called anti-idiotypes are very specific and resemble antigen in their selectivity in that they only react with a particular subset of antibody combining sites which has light and heavy chains of the proper sequence. The Fab fragment is formed of light chain plus the so-called Fd piece of the heavy chain. This fragment contains the combining site for antigen which is formed by interaction between the variable region of the light chain and the variable region of the heavy chain. We have produced these types of antisera against immunoglobulins and isolated T cell products of murine and human, as well as other primates.

It is possible to use these anti-combining site reagents to detect immunoglobulin related components on the surface of certain T cells of normal individuals and on certain *in vitro* grown T cell leukemia or lymphoma lines. Fig. 4 illustrates immunocytofluorescence data comparing the binding of chicken anti-mouse Fab (shotgun) developed with fluorescein-labeled rabbit anti-chicken immunoglobulin (B,D) and the binding by one of the cells of rodamine labeled myoglobin (A) or horse spleen ferritin (C). The usual frequency for binding of protein antigens by T cells of unimmunized animals is about one per thousand T cells (DeLuca et al., 1979). It is an important observation here that the distribution of antigen on the cell surface in A and B and C and D is the same as the distribution of the immunoglobulin. This result is expected if the immunoglobulin serves as the primary binding receptor for the antigen. We have carried out a series of studies in which there was an absolute correspondence between antigen Fab-related determinant using a variety of antigens and counting close to fifty individual cells (DeLuca et al., 1979). This sort of observation provides strong presumptive evidence that the immunoglobulin-like molecule

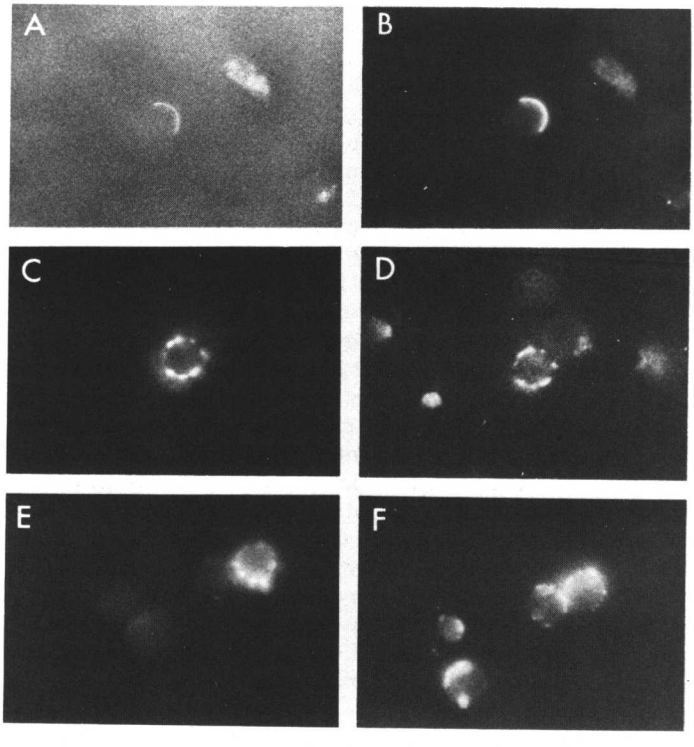

Fig. 4. Codistribution of rodamine-labeled horse spleen ferritin (A) and chicken anti-mouse Fab plus fluorescein-labeled rabbit anti-chicken immunoglobulin (B) on a thymic antigen binding cell. Codistribution of rodamine-labeled keyhole limpit hemocyanin (C) and chicken anti-mouse Fab plus fluorescein-labeled rabbit antibodies to chicken IgG (D) on a second thymic antigen binding T cell. Photographs E and F show the lack of codistribution of spots of rodamine-labeled keyhole limpit hemocyanin and antibody to histocompatibility antigen ($H\text{-}2^d$) plus fluorescein-labeled rabbit anti-mouse immunoglobulin respectively. Since the anti-$H\text{-}2^d$ is detected by fluorescein-labeled rabbit anti-mouse Ig, the lack of codistribution shown in E and F also indicates that the thymic antigen binding cells are T cells rather than B cells (x1500). (Data of DeLuca et al., 1979).

is the primary receptor for antigen on T cells. By contrast in control experiments, neither the Thy-1 alloantigen nor the H-2 histocompatibility antigens codistributed with foreign antigens (E and F). Thus, the evidence indicates that these molecules are not primary receptors for antigen.

We further approached the problem of specificity by comparing our rabbit anti-idiotype with our shotgun chicken anti-Fab for their capacities to inhibit the binding of serum antibody to the arsonate hapten and also the binding of the arsonate hapten by antigen specific T cells isolated from immunized strain A/J mice (Warr et al., 1979). Fig. 5 presents data obtained in experiments blocking binding of the idiotype bearing antibodies to the hapten in solution. It can be seen that the binding of hapten by the combining site of the idiotype-bearing molecule is blocked by anti-idiotype but not by normal rabbit serum or by rabbit antiserum directed against the constant region of the heavy chain (anti-IgG 2). This is the expected result and confirms that our anti-idiotype, which is directed against the V_H/V_L interaction determinant, reacts with the combining site. In addition, our "shotgun" anti-Fab also is a very effective inhibitor of blocking of hapten binding by the idiotype-bearing molecule. These data do not indicate that the "shotgun" and the anti-idiotype compete for precisely the same determinants on the antibody. They do, however, establish that both compete for the same general region of the molecule. The parallel experiment was performed using antigen-binding T cells in which the arsonate hapten was coupled to fluorescein-labeled bovine serum albumin and the assay was performed by cytofluorescence. Approximately 2-4% of peripheral T lymphocytes of the immunized A/J mice bound the antigen specifically as shown in Fig. 6. The specificity of the binding for the arsonate hapten is shown by the virtually complete inhibition by arsonate-derivatized bovine serum albumin coupled with the fact that bovine serum albumin alone does not inhibit binding at all. The rabbit anti-idiotype showed substantial blocking up to approximately 80%, a value which is expected because approximately 70-80% of antibodies to the arsonate hapten bear the crossreactive idiotype (Ju et al., 1977). The chicken anti-Fab gave total inhibition of the reaction, and the specificity of this is shown because it was possible to inhibit the blocking by titrating in normal polyclonal mouse immunoglobulin which bore the collection of Fab region determinants. This evidence, thus, indicates that antigen specific T cells express an Fab-related surface molecule which bears immunoglobulin idiotype and is also detectable using chicken antibodies directed against combining site determinants found on pooled murine immunoglobulin.

Is the Ig-related T cell product identical to serum antibodies or to B cell surface immunoglobulins? This has been an extremely contentious issue and many papers have been written disputing this

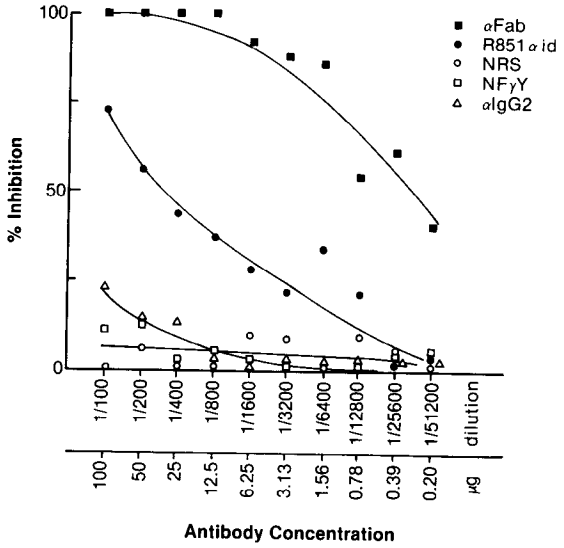

Fig. 5. Inhibition of the binding of idiotype-bearing anti-ARS antibody to the ARS hapten. Anti- (Fab), chicken antibody to the $(Fab')_2$ fragment of mouse IgG; R815, anti-idiotypic antiserum to the idiotypic determinants of the murine anti-arsonate (ARS) antibody; NRS, normal rabbit serum; anti-IgG_2, rabbit anti-serum to mouse IgG_{2a} and IgG_{2b} (these are the class specific constant region determinants of the idiotype bearing IgG antibodies studied here); NFγY, normal chicken IgY. The dilution scale refers to the intact sera, the microgram scale refers to the anti-$(Fab')_2$ and the normal chicken IgY, both of which are purified immunoglobulins. (Data of Warr et al., 1979).

point. The most direct way to approach the problem is to isolate the T cell product and to compare them directly with the B cell immunoglobulins by serological and biochemical techniques. When this is done, it is clear that the answer to this question is "no". Fig. 7 illustrates the idiotype-positive molecule synthesized by peripheral T cells of A/J mice immunized to the arsonate hapten. The only significant component detectable has an approximate mass of 67,000 daltons. The peripheral T cells were stimulated by the T cell mitogen Concanavalin A prior to pulse labeling with ^{75}Se-methionine. A similar preparation utilizing purified B cells from the same animal was negative for the presence of componants in the 67,000 dalton range. The existence of a T cell derived molecule which bears antigenic determinants related to those of

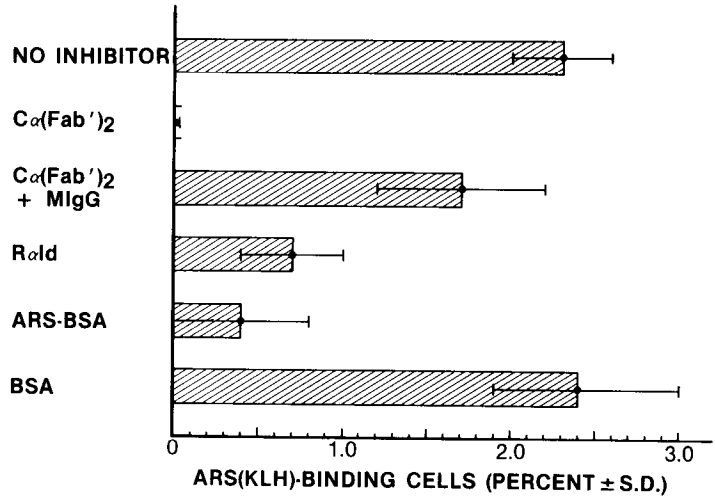

Fig. 6. Inhibition of binding of the arsonate (ARS) hapten by peripheral T cells of primed A/J mice by anti-idiotype and by chicken anti-Fab reagents. The arsonate hapten was coupled to rodamine-labeled keyhole limpit hemocyanin for the use in these binding studies. The specificity of the reaction for the arsonate hapten is illustrated by the fact that ARS coupled to bovine serum albumin (ARS-BSA) inhibits binding whereas the BSA carrier alone does not. The reaction is blocked to about 80% by rabbit anti-idiotye (RαID) and is completely inhibited with the chicken anti-mouse Fab fragment. Addition of purified murine IgG immunoglobulin (MIgG) reverses the inhibition by the chicken anti-Fab, thereby indicating the specificity of the inhibition reaction. (Based upon data of Warr et al., 1979).

immunoglobulin combining sites is further illustrated in Fig. 8 which presents a fluorograph obtained for biosynthesis of a V_H-bearing molecule by a long-term marmoset amplifier T cell line 70-N2. Although some material is present in the control, and most probably represents actin or proteins which bind to the Fc fragment of immunoglobulin. It is noteworthy that appreciable amounts of material resembling light polypeptide chains in SDS gel mobility are absent in both the mouse and the primate T cell biosynthetically labeled product (Marchalonis et al., 1980b). In some cases, we and others have observed polypeptide chains resembling light chains to