Visualization of a Guinea Pig T Lymphocyte Surface Component Cross-Reactive with Immunoglobulin

Abstract. *Thymus-derived lymphocytes (T cells) show exquisite specificity in recognition of antigens, but the nature of the cell surface receptor is controversial. Although antigen recognition mediated by immunoglobulin variable (V) regions remains the minimal hypothesis, it has been extremely difficult to definitely establish the presence of immunoglobulins on these cells. Chicken antibodies, produced against the (Fab')$_2$ fragment of mouse immunoglobulin G (IgG) and purified by binding to and elution from IgG-Sepharose 4B, bind to an endogenously synthesized surface component of guinea pig T cells. The binding occurred via a cross-reaction with murine κ chain and a heavy chain determinant localized in the Fd region, and was visualized by immunofluorescence and immunoelectronmicroscopy using both transmission and scanning techniques. These data provide direct evidence for the presence of a surface component related to immunoglobulin on T lymphocytes.*

Two broad classes of functionally distinct lymphocytes exist; namely, thymus-derived lymphocytes (T cells), which mediate cellular immune responses including destruction of tumors, and bone-marrow–derived lymphocytes (B cells), which are the precursors of antibody-forming cells (*1*). Cells of both classes exhibit exquisite specificity in the recognition of antigen. It is generally accepted that the surface receptor for antigen on B cells is immunoglobulin (*2*), but the nature of the antigen receptor on T cells has been the subject of controversy. The minimal hypothesis is that the T cell receptor is also an immunoglobulin-like molecule that mimics antibody in its specificity for antigen (*3, 4*). However, attempts to establish the existence of endogenously synthesized surface immunoglobulin on T lymphocytes have been fraught with technical and conceptual difficulties. Although numerous investigators have isolated an immunoglobulin-like surface receptor molecule from mammalian T lymphocytes (*3–5*), it has been extremely difficult to visualize these molecules directly (*2*). By contrast, immunofluorescence assays with mammalian antiserums to serum immunoglobulins of lower vertebrates show that virtually all lymphocytes of these species (*6*), including T-type helper cells (*7*), bear readily detectable surface immunoglobulin molecules. The opposite approach, incorporating the principle of raising antiserums to immunoglobulins in phylogenetically distant species (which might detect cross-reactions between B cell–type and T cell–type immunoglobulins), has also been applied successfully with the use of avian antibodies to demonstrate endogenously produced surface immunoglobulins of murine (*8, 9*) and human (*10*) T cells. Moreover, antibodies directed against immunoglobulin variable region antigenic determinants (idiotypes) bind directly to T cells (*4*).

In this report, we use the principle of raising antibodies in a phylogenetically distant species to demonstrate the presence of an endogenously synthesized immunoglobulin-like molecule on the surface of guinea pig T cells. In order to increase the likelihood that our antibodies would react with variable region determinants, we used as immunogen the (Fab')$_2$ fragment of normal mouse immunoglobulin G (IgG). This fragment contains the antigen-combining site and comprises light chains (predominantly κ chains) and the Fd piece of the heavy chain, which contains the variable (V_H) region. Chicken antibodies raised in this manner cross react with guinea pig immunoglobulins (*9*). Studies were carried out with guinea pig T cells because this species exhibits extremely vigorous T cell responses that have provided important models for cell-mediated immunity (*11*). We report here that purified chicken antibodies to the (Fab')$_2$ fragment of mouse immunoglobulin recognize a surface molecule on guinea pig T cells, which cross reacts with murine immunoglobulin (Ig) determinants. We have been able to show the surface localization of the T cell immunoglobulin-like material by utilizing high-resolution immunoelectronmicroscopy as previously described for the detection of surface immunoglobulin on lymphocytes (*12*). These studies constitute the first demonstration of endogenous immunoglobulin on guinea pig T cells.

Chicken antibodies were raised against the (Fab')$_2$ fragment of mouse IgG and were isolated by binding to and elution from IgG-Sepharose immunoadsorbents (*9*). We tested these antibodies for their capacity to bind to guinea pig lymphocytes by means of indirect fluorescence, with the use of fluorescein-labeled rabbit antibodies to chicken immunoglobulin as the developing reagent. Guinea pig T cell suspensions were prepared from thymus glands of young adult males of strain 2 guinea pigs. The thymus was teased with hypodermic needles in Eagle's minimal essential medium (EMEM), and the cell suspension was filtered through a nylon bag. The cells were washed and incubated in a glass wool column at 37°C for 30 minutes and then eluted with EMEM. On the basis of an immunofluorescence study with fluorescein-conjugated rabbit antibody to guinea pig gamma globulin (Cappel Laboratories), it was shown that the eluate contained less than 2 percent of B cells.

Guinea pig T cells were incubated with various concentrations of chicken antibody to mouse (Fab')$_2$ and then with either fluorescein- or ferritin-conjugated rabbit antiserum to chicken gamma globulin (Cappel Laboratories). Immunofluorescence was assessed by direct inspection and by quantitative measurement of individual cells by means of a Zeiss photomicroscope III equipped with a photometer. Fluorescence intensities of 100 to 200 cells from each group were measured. To minimize the exposure of cells to ultraviolet light, we measured only two to three cells per microscope field. Single cells were centered in the field under white light, and fluorescence was measured during a 2-second pulse of ultraviolet light. Fluorescence intensity was a function of anti-

Table 1. Distribution of Ig-like material on the guinea pig T-cell surface, visualized after the addition of chicken antibodies to mouse Fab plus fluorescein-labeled rabbit antiserum to chicken IgY.

Distribution	Percentage of cells after					
	First labeling			Second labeling		
	0 hour	1 hour	6 hours	2 hours	4 hours	6 hours
No fluorescence	5*	14	12	20	6	7
Strong ring fluorescence	63	0	0	8	25	32
Weak ring fluorescence	32	0	0	5	11	24
Patches	0	61	0	26	13	2
Caps	0	25	88	32	21	5
Caps and rings	0	0	0	9	24	30

*At each time point 100 to 200 cells were counted.

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body concentration. Quantitative estimates of relative fluorescence intensity showed, for example, that 2.5 μg of chicken antibody gave a mean (± S.E.) photometer reading of 46 ± 2.2 units, whereas 10 μg of antibody gave a mean of 147 ± 6.4 units. All values of the background fluorescence obtained with normal chicken immunoglobulin Y (IgY) at these concentrations plus fluorescein-labeled rabbit antibody were between 10 and 20 photometer units. Visual inspec-

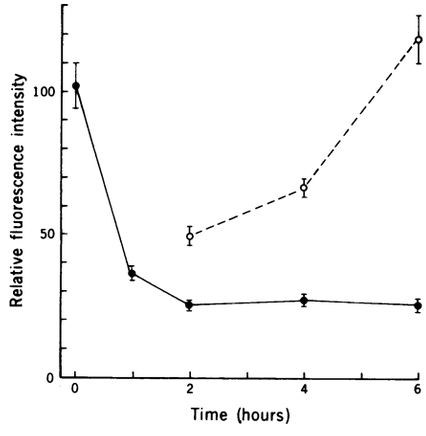
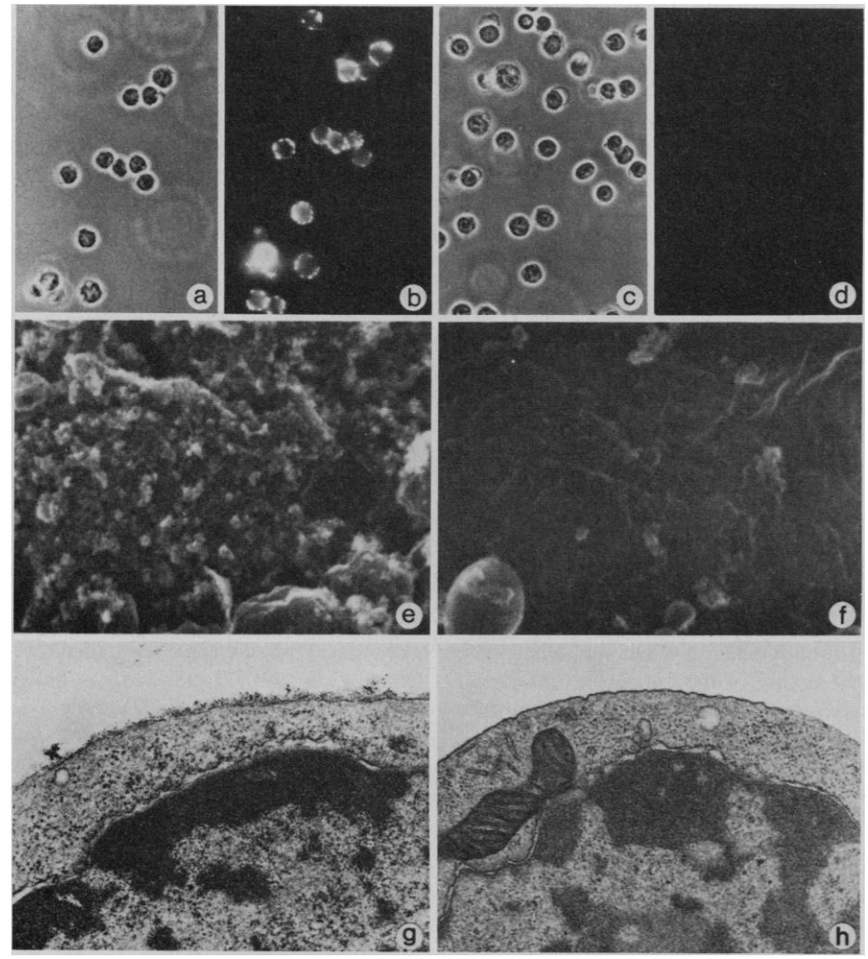
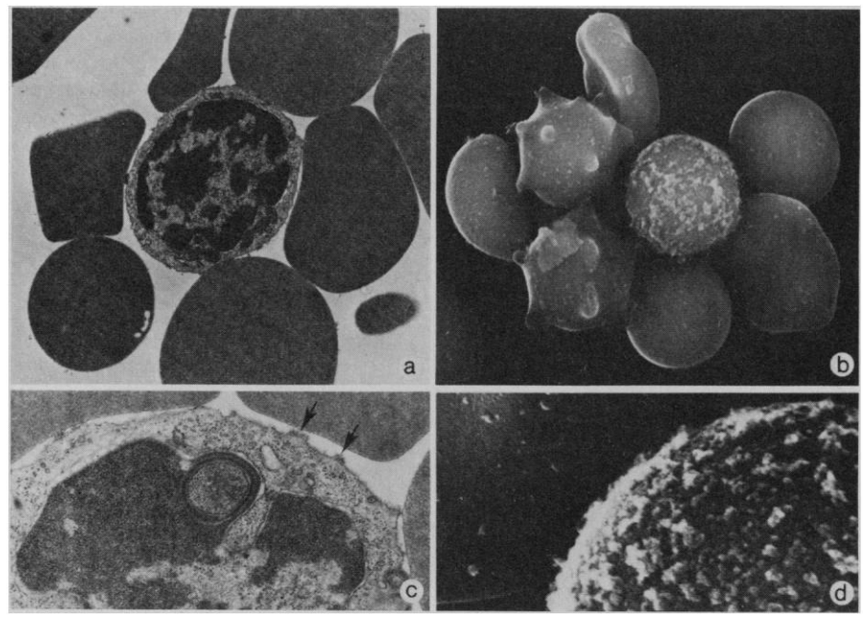


Fig. 1 (left). Loss and reappearance of κ-like surface material of guinea pig thymic T cells as assessed by quantitative immunofluorescence analysis; ordinate, photometer readings, mean ± standard error: abscissa, time of incubation at 37°C. (●——●) Loss of initial fluorescent label. (○------○) Reappearance of κ-like material demonstrated by readdition of labeled reagents at times indicated. Immunofluorescence analysis was performed as described (9). Chicken antibody (5 μg) was added to 5×10^6 cells. Fluorescence was developed by addition of 30 μg of the γ-globulin fraction of fluorescein-labeled rabbit antibody (10.4 mg of total protein per milliliter; 2.5 mg of specific antibody per milliliter; Cappel Laboratories). Fluorescence of approximately 100 individual cells in each group was measured with the Zeiss photomicroscope III and photometer attachment. Fig. 2 (top right). Guinea pig thymic T cells were incubated at 4°C with 10 μg of chicken antibody to Fab followed by either fluorescein-conjugated rabbit antibody to chicken IgY or ferritin-conjugated rabbit antibody to chicken IgY. Control samples were incubated with normal chicken serum followed by labeled antibody. Phase and fluorescence micrographs of guinea pig T cells are shown in (a) and (b) and the controls are shown in (c) and (d) (× 675). Scanning electron micrograph of guinea pig T cells with labeled ferritin is shown in (e) and the corresponding control in (f) (× 40,000). Transmission electron micrograph of ferritin-labeled guinea pig T cell is shown in (g) and its control in (h) (× 34,000). Phase and fluorescence micrographs were taken on a Zeiss photomicroscope III. Samples processed for TEM and SEM were incubated with 10 μg of chicken antibody followed by a 1:4 dilution of ferritin-conjugated rabbit antibody to chicken IgY (Cappel Laboratories). Fig. 3 (bottom right). Guinea pig T cells were incubated at 4°C with 4 μg of chicken antibody to Fab for 30 minutes, washed, and then rosetted with rabbit red cells for 4 hours; the rosettes were washed and incubated with ferritin-conjugated rabbit antibody to chicken γ-globulin, and processed for SEM or TEM. The TEM of a rosetted T cell containing labeled ferritin on its surface is shown in (a) (× 9,000) and a higher magnification showing patches of ferritin as well as contact areas between T cells and rabbit red cells are shown in (c) (× 22,000). The SEM of a rosette with ferritin-labeled T cell is shown in (b) (× 7,250), and a higher magnification of T cell with red cell is shown in (d) (× 32,000). A reciprocal experiment was also done where the T cells were rosetted first and then incubated with chicken antibody to Fab and ferritin-conjugated antibody. The labeling pattern of the T cells appeared similar to those shown above.





tion disclosed that more than 95 percent of the cells were brightly fluorescent with the chicken antiserum to Fab reagent (Fig. 2).

Evidence of the specificity of the reaction was obtained by means of absorptions with Sepharose alone and various murine immunoglobulins coupled covalently to this matrix. The immunoglobulins used were the λ light chain produced by the myeloma RPC 20, the κ light chain produced by the myeloma MOPC 41, and intact normal mouse immunoglobulin of the IgG_{2a} and IgG_{2b} classes. Fluorescence was not markedly diminished by absorption of the antibody with λ-chain Sepharose or by Sepharose alone, but was substantially reduced by absorption with κ-chain Sepharose and completely eliminated by absorption with IgG-Sepharose. These results show that the binding of chicken antiserum to mouse Fab to guinea pig cells parallels that reported for binding of this antiserum to murine T and B cells (*9*) where the reaction is largely directed against κ determinants, but with heavy chain (Fd) determinants also contributing.

Immunofluorescence analysis was used to obtain evidence that the Ig-like material was produced by the guinea pig T cells. The living, labeled cells were incubated in EMEM at 37°C and the distribution of label was monitored by immunofluorescence at intervals during incubation. The label was initially distributed uniformly about the cell periphery (ring), but redistributed into patches and caps (Table 1). By 6 hours all detectable label was localized in small caps. Figure 1 provides a quantitative assessment of the loss of surface label with time of incubation. A second addition of labeled reagents indicates that, after 6 hours of incubation, the Ig-like material was regenerated, both quantitatively (Fig. 1) and in terms of uniform distribution of the rings (Table 1).

Ferritin-labeled rabbit antibody to chicken immunoglobulin was the developing reagent in immunoelectronmicroscopic studies. Samples for immunoferritin-labeling of T cells were fixed in 2.5 percent glutaraldehyde in cacodylate buffer and processed for transmission electron microscopy (TEM) (*13*). Samples for scanning electron microscopy (SEM) were fixed with osmium tetroxide, dehydrated through a graded concentration series of ethanol, critical-point dried through Freon 13, coated with platinum-paladium alloy, and examined in a field emission scanning electron microscope (Hitachi HFS-2; operating at 25 kv). Criteria for the identification of ferritin-labeled cells by SEM were: (i) the demonstration of clustered ferritin on the cell surface, (ii) a comparison of immunoferritin-labeled cells with nonlabeled controls, and (iii) correlation of results with SEM and TEM (Fig. 2). With high concentrations of antiserum (10 μg) a continuous layer of ferritin-labeled antibody was observed external to the plasma membrane in TEM, and as a granular layer when analyzed in SEM (Fig. 2).

Although the T lymphocyte preparation studied above was depleted of B cells and adherent cells by passage through glass wool and more than 95 percent of the cells were positive for immunoglobulin when the chicken reagent was used, we performed experiments to determine whether cells bearing T markers expressed immunoglobulin cross-reactive determinants. It was previously shown that guinea pig T cells form spontaneous rosettes with rabbit erythrocytes (*14*), and we wished to determine whether the rosetting lymphocytes bind with chicken antiserum to mouse Fab. Such cells were positive by immunofluorescence analysis and exhibited immunoferritin-label (Fig. 3) as assessed by TEM and SEM.

We conclude that guinea pig T lymphocytes express an endogenously generated surface component, which at least cross reacts with murine immunoglobulin. Probably the cross-reaction is apparent because antiserums were raised in an avian species, an offshoot of dinosaurs (*15*), which diverged early from the line leading to mammals. As previously reported (*9*), chicken antiserum to mouse Fab is a reagent that binds to rodent immunoglobulins, but not to those of other vertebrate classes such as primates. These antibodies, which bind to virtually all guinea pig T and B cells, react with both κ chain determinants and heavy chain determinants localized in the Fd region, but the exact binding sites have not yet been established. The reaction is neither with carbohydrate determinants nor alloantigens because (i) absorption with the κ-chain MOPC 41, a polypeptide of known sequence (*16*), substantially reduces binding, (ii) only lymphocytes bear the antigenic determimant (*9*, *17*), and (iii) absorption of the antibody to Fab with nonlymphoid cells bearing histocompatibility antigens did not remove binding to T lymphocytes. Because this component can now be visualized, experiments may now be designed to determine whether this Ig-like molecule is involved in primary union with antigen. Moreover, the use of immunoelectronmicroscopic technology will facilitate studies directed toward obtaining objective estimations of the distributions of immunoglobulins of T as well as B cells. Results of these studies will bear directly upon the nature of the T cell receptor involved in primary union with antigen and the mechanisms by which this binding initiates specific immune differentiation.

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17. The chicken antiserum to Fab was found to be negative when tested against rodent erythrocytes, monocytes, fibroblasts, and various cultured tumor cells including melanomas, fibrosarcomas, mastocytomas, and myeloid leukemias. Because the purified antibody was isolated with a Sepharose-based immunoabsorbent, some preparations contained naturally occurring chicken antibodies directed against carbohydrates cross-reactive with the matrix. Such antibodies bound to fibroblasts. This spurious reactivity was removed by passage of the purified antibody through Sepharose.
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