anisms) which maintains a high amount of potassium in the fetus, the role of this ion in fetal development, and the possible general applicability of the concept of hypopotassemia as a cause of fetal kidney maldevelopment demand further study.

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Antigen Receptor Molecules: Inhibition by Antiserum against Kappa Light Chains

Abstract. *Rabbit antiserum against mouse kappa chains, the predominant class of light chains found in mouse immunoglobulins, inhibits the immune response in vitro of mouse spleen cells to erythrocyte antigens. The inhibition becomes irreversible if the mouse spleen cells are treated briefly with both antiserum to kappa chains and complement.*

It is generally believed that certain lymphoid cells in immune responses carry antibody-like molecules on their surfaces and are responsible for specific antigen recognition. Sell and Gell, as reviewed by Gell (*1*), have presented ample evidence for the presence of immunoglobulin molcules on lymphoid cell surfaces; they used rabbit antiserum to rabbit immunoglobulin allotypes or sheep antiserum to rabbit light or heavy chains to stimulate blast transformation and DNA synthesis in rabbit lymphocytes. But their experiments do not establish any function of the immunoglobulin-bearing cells with respect to antigen recognition. Greaves, Torrigiani, and Roitt (*2*) have suggested a role for immunoglobulin-carrying lymphocytes in cell-mediated immune responses in vitro. The delayed hypersensitivity and homograft responses of human lymphocytes in vitro to tuberculin antigen (purified protein derivative) and histocompatability antigens, respectively, could be inhibited by the Fab (antigen binding) fragment of antibody against the light chain component of human immunoglobulin. They proposed that antibody to light chain binds to sites on the lymphocyte surface and blocks combination with antigen either by steric hindrance or by causing a configurational change in the receptor site. It is not known whether the immunoglobulins involved in antigen recognition contain both heavy and light chains or, if they contain heavy chains, whether they are of a known heavy chain class. We present evidence that mouse immunoglobulin molecules that contain light chains play a part in the generation of plaque-forming cells in response to heterologous eythrocytes in mouse spleen cell suspension cultures.

Suspensions of normal mouse spleen cells from BDF_1 (C57B1/6 × DBA/2) mice were cultured in a medium containing 5 percent fetal bovine serum (*3*). Thirty microliters of a 1 percent suspension of the erythrocyte antigen (about 3×10^6 cells) was added to each culture dish containing 1 ml of cell suspension at the density indicated. The number of spleen cells forming 19*S* antibody against sheep (S-RBC) or horse (H-RBC) erythrocytes was assayed after 4 days of culture by a modification (*3*) of the Jerne *et al.* plaque technique (*4*). Rabbit serum (5 percent) was added to cultures at time zero. This was either normal rabbit serum (NRS) from unimmunized rabbits, or rabbit antiserum to a mouse kappa chain obtained from mouse myeloma protein (*5*). The rabbit serums were extensively absorbed with sheep erythrocytes and filtered through a washed 0.22-μm Millipore filter. Normal mouse serum (NMS) was prepared from pools of blood collected from about 20 male and female BDF_1 mice; it was also absorbed with sheep erythrocytes and passed through a Millipore filter.

Table 1. Inhibition of in vitro response by antiserums to mouse kappa chains (anti-κ). Serums were added to cultures at time zero. Sheep erythrocytes were added at 1 hour. Results are given as the number of plaque-forming cells (PFC) per 10^6 cells recovered at day 4.

Additions	PFC at cell densities (cell/ml):		
	12×10^6	8×10^6	4×10^6
None (control)	897	754	554
5% anti-κ	32	5	0
5% anti-κ + 3% NMS	1446	532	426
5% NRS	1070	651	413
5% NRS + 3% NMS	1008	455	67
5% anti-κ at end	1103	733	654

Rabbit antiserum to mouse kappa chains, present throughout the culture period at a 1 : 20 dilution, inhibited the plaque-forming cell response to S-RBC almost completely. Normal rabbit serum in the same concentration stimulated cultures, so that the response was greater than that seen with 5 percent fetal bovine serum alone (Table 1). At the highest cell density in the cultures (1.2×10^7 cell/ml) inhibition was sometimes incomplete with 5 percent antiserum. Since higher serum concentrations were toxic to cultures, complete inhibition was obtained with spleen cell cultures of lower densities.

The addition of 3 percent normal mouse serum to the cultures was sufficient to reverse completely the inhibition by the antiserum to kappa chains, presumably by the binding of antibody against kappa chains to immunoglobulins in the mouse serum. The higher serum concentration (3 percent NMS plus 5 percent rabbit serum) was somewhat toxic, especially in cultures at low cell densities, giving low total cell yields and low plaque-forming cell yields as compared to yields from cultures with less serum added. In concentrations high enough to neutralize the antiserum to kappa chains, NMS is able to restore activity to inhibited cultures, the subsequent response being equal to that in NRS plus NMS control cultures.

Fuji and Jerne (*6*) and our colleagues, Hartmann *et al.* (*7*), have obtained very similar results with other

Table 2. Reversal of inhibition on removal of antiserum to kappa chains (anti-κ). Mouse spleen cells at the density indicated were incubated at 37°C with or without 5 percent anti-κ. After 1 hour, cells were resuspended in fresh medium or in the same medium for culture and 3×10^6 S-RBC were added. Results are given as the number of plaque-forming cells per 10^6 cells recovered at day 4. N.D., not done.

Additions	PFC at cell densities (cell/ml):		
	12×10^6	8×10^6	4×10^6
None (control)	701	N.D.	N.D.
Anti-κ to end	17	1	1
Anti-κ, 1 hour	1086	475	125

antiserums to mouse immunoglobulins. The latter have shown that antiserum to IgG, antiserum to Fab (γ2a) and antiserum to IgM are inhibitory but that antiserum to Fc (γ2a) and antiserum to the heavy chain allotype are not inhibitory.

Addition of rabbit antiserum to mouse kappa chains at the end of the culture period (last line, Table 1) did not interfere with the detection of plaque-forming cells. This indicated that the antiserum to kappa chains does not inhibit simply by inhibiting cells that have already been stimulated to produce antibody.

These data show that the specific binding of the rabbit antibody against mouse kappa chains to mouse kappa molecules in the cultures prevents the stimulation of an immune response by antigen. The mechanism of this inhibition is not known. The results suggest that rabbit antiserum to kappa chains interferes with mouse kappa chains involved in antigen recognition. Binding of rabbit antibody to kappa molecules, either free or on cell surfaces, might be expected to prevent the interaction between specifically sensitive cells and antigen.

If mouse spleen cells are incubated with antiserum to kappa chains for 1 hour, centrifuged, and suspended in fresh culture medium that contains no antiserum to kappa chains, there is no inhibition of plaque-forming cells (Table 2). If the antibody to kappa chains was indeed initially bound to cells with kappa chains on their surfaces, it would appear that the amount of bound antibody retained is insufficient to maintain inhibition over the 4-day culture period. This could be due either to bound antibody to kappa chains dissociating from the cell surfaces or to cells dividing or synthesizing new surface kappa chains that are not blocked by antibody (or both). However, if spleen cells are incubated at 37°C in the presence of antiserum to kappa chains and complement, and then are washed and cultured, the response to antigen is completely inhibited. This is shown in Table 3 for responses to both horse and sheep erythrocytes. Neutralizing the antiserum to kappa chains by the simultaneous addition of NMS prevents the inhibition by antiserum to kappa chains and complement. It is not known whether the cells that survive treatment with antibody to kappa chains to give plaque-forming cells at 4 days represent (i) rare cells bearing only lambda light chain molecules, or (ii) kappa chain–bearing cells that are not destroyed by the treatment.

Table 3. Irreversible inhibition after brief treatment with antiserum to kappa chains and complement. Mouse spleen cells were incubated at 1.2×10^8 cell/ml with antiserum to kappa chains at 0°C for 30 minutes. Guinea pig serum was added, and the cells were incubated for 15 minutes at 0°C and 20 minutes at 37°C. The cells were resuspended in fresh medium before culture with antigen. Amounts used: 0.5 ml of spleen cells, 0.25 ml each of antiserum to kappa chains, normal mouse serum (NMS) and complement (C′). Values are given as plaque-forming cells per 10^6 recovered cells measured on day 4.

Prior treatment	PFC (No.)			
	S-RBC response at (cell/ml):		H-RBC response at (cell/ml):	
	12×10^6	6×10^6	12×10^6	6×10^6
Cells only	1077	1005	273	263
Anti-κ + C′	7	11	0	2
Anti-κ + NMS + C′	859	635	148	286

Treatment with complement apparently kills or in some other way inactivates those cells that have bound antibody to kappa chains. The ability of antiserum against kappa chains to inactivate spleen cell populations irreversibly in the presence of complement indicates that the antibody has bound to the surfaces of cells that are necessary at some step in the generation of a plaque-forming cell response. This conclusion is reinforced by the fact that removal of excess free antiserum to kappa chain before addition of complement, with only what is cell-associated being left, still results in destruction of responsive cells, though the destruction is less complete than that caused by simultaneous treatment with complement and excess antiserum to kappa chains. This experiment establishes that there are cells which bear on their surfaces kappa chains that are needed for an immune response against erythrocytes in culture. The mechanism of inhibition of the response by prior treatment with antiserum and complement is not necessarily the same as the inhibition by the presence of antiserum in culture (Table 1) and may not be affecting the same essential step in the response. Inhibition of a response by the presence of antiserum in culture (Table 1) may occur by the blocking of kappa chains which are either free in culture or bound to the surface of some cell type. Inhibition by prior treatment of spleen cells with antiserum and complement (Table 3) must occur by the destruction or inactivation of a cell bearing kappa chain molecules on its surface. In neither experiment is inhibition shown to be due to interference with antigen recognition, although such a mechanism might be postulated.

The use of specific antiserum against mouse immunoglobulin components offers a way of studying antibody-like molecules whose function may be specific recognition and binding of antigen. Although this system is not antigen specific, it does provide a means of studying the classes of immunoglobulin-like molecules that are involved in the stimulation of an immune response. The ability to deplete a spleen cell population of immunoglobulin bearing cells by treatment with specific antiserum and complement should facilitate study of the function and cell interactions of these cells in immune responses.

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