Polypeptide Chains of Human Gamma-Globulin: Cellular Localization by Fluorescent Antibody

Abstract. *Fluorescent antibody to antigenically distinct portions of 7S gamma-globulin was used to study the occurrence of H-chains and L-chains of types I and II within single cells. Individual lymphoid cells contained both H- and L-chains, but individual cells did not contain both antigenic types of L-chains.*

Mammalian 7*S* γ-globulin (γ_2-globulin) molecules have been shown to have at least two kinds of polypeptide chains (*1*). In the human globulin, these chains are termed H (for Heavy) and L (for Light). The H-chain possesses the antigenic and structural specificities of 7*S* γ-globulin; the L-chains are moieties common to all of the immunoglobulins (7*S* γ-, β_{2A}-, and β_{2M}-globulins) and are closely related to Bence-Jones proteins (*2, 3*). Like Bence-Jones proteins, L-chains (and their parent globulin molecules) may be grouped into two broad antigenic classes; type I and type II (*3*). In the serums of normal individuals, the ratio of type I immunoglobulins to type II immunoglobulins is approximately 2:1 (*3, 4*). The 7*S* γ-globulin paraprotein present in the serum of a myeloma patient is of only one antigenic type. Type I paraproteins are encountered twice as frequently as type II paraproteins in such patients (*5*).

In our study, Coons' fluorescent-antibody technique (*6*) was used to determine whether single cells produce more than one kind of 7*S* γ-globulin polypeptide chain at one time. The polypeptide chains investigated were L-chains of types I and II, and the H-chain of 7*S* γ-globulin. This study was made possible by the availability of antiserums highly specific for each of the chains of 7*S* γ-globulin and by improvements in the preparation and use of fluorescent antibody (*7*).

Antiserums were produced in male New Zealand rabbits. The antigens used for immunization were two highly purified Bence-Jones proteins (Ag, of type I, and Bo, of type II) (*8*) and the highly purified urinary protein (Cr) excreted by a patient with a lymphoma-like disease described by Franklin (*9*). Antiserums to these proteins have these specificities: Antiserum to Ag reacts with antigenic type I molecules (7*S* γ, β_{2A}-, β_{2M}-globulins, and Bence-Jones proteins) but not with those of type II; antiserum to Bo reacts with antigenic type II molecules but not with those of type I; antiserum to Cr reacts with 7*S* γ-globulin and with the papain-produced F fragment; it does not react with the S fragments of 7*S* γ-globulins, with β_{2A}-globulins, β_{2M}-globulins, or with Bence-Jones proteins. The three antiserums were used without absorption and do not cross-react with each other.

The immune γ-globulins were isolated from these antiserums by ammonium sulfate precipitation and diethylaminoethyl cellulose chromatography. Tetramethylrhodamine isothiocyanate or fluorescein isothiocyanate were conjugated to the rabbit immune γ-globulin preparation at *p*H 9.3 in the cold. Optimum ratios of dye to protein were obtained by chromatography on diethylaminoethyl cellulose, according to the methods of Goldstein *et al.* (*7*) with some modifications and certain adaptations for preparation of rhodamine derivatives (*10*). Gel filtration of the rhodamine conjugates on Sephadex G-50 preceded ion-exchange chromatography. Fractions were concentrated by pressure dialysis.

Four fluorescent conjugates were prepared, namely, fluorescein-conjugated antibody to Cr (termed Green-Cr), rhodamine-conjugated antibody to Ag (Red-I), fluorescein-conjugated antibody to Bo (Green-II), and rhodamine-conjugated antibody to Bo (Red-II). Staining by all conjugates used in the experiments could be specifically blocked by the prior application of a 10- to 30-fold concentration of the homologous nonconjugated immune globulin to the specimen. The optimal concentration of each fluorescent conjugate was determined in a series of preliminary experiments and the reagents were used at the optimal concentration throughout the experiments reported. The protein concentrations used and the ratio of optical densities at 280 mμ and 550μ for rhodamine conjugates and at 280 mμ and 495 mμ for fluorescein conjugates were as follows: Red-I (3 mg/ml), 3:1; Red-II (1 mg/ml), 5:1; Green-II (0.15 mg/ml), 2:1; and Green-Cr (0.5 mg/ml), 3.3:1. Although the Red-II and Green-II conjugates were prepared

Table 1. Percentages of stained cells in various lymphoid tissue preparations showing red, green, or mixed fluorescence.

Cell color	Tissue specimen						
	Spleen No. 1	Lymph Node No. 1	Spleen No. 2	Spleen No. 3	Lymph Node No. 3	Spleen No. 4	Lymph Node No. 4
	Experiment 1. Red-I (L-I) and Green-II (L-II)						
Red	60		69	66	68	66	61
Green	39	*	30	32	31	33	36
Mixed	1		1	2	1	1	3
	Experiment 2. Red-I (L-I) and Green-Cr (H)						
Red	30	40	34	28	24	36	34
Green	27	19	32	25	23	15	23
Mixed	43	41	34	47	53	49	43
(M/G+M)×100†	61	69	51	65	69	75	67
	Experiment 3. Red-II (L-II) and Green-Cr (H)						
Red		32	17	20	28	36	13
Green	*	41	53	39	43	41	47
Mixed		27	30	41	29	23	40
(M/G+M)×100†		39	37	52	40	36	46
	Experiment 4. Red-I (L-I) and Red-II (L-II) and Green-Cr (H)						
Red		49	25	27	35	47	33
Green	*	2	8	0	6	2	5
Mixed		49	67	73	59	51	62
	Experiment 5. Red-II (L-II) and Green-II (L-II)						
Red	2		2		0	0	
Green	0	*	0	*	0	0	*
Mixed	98		98		100	100	

* Not done. † (M/G+M)×100 = percent of mixed cells relative to total green plus mixed cells.

from the same immunoglobulin preparation, the Red-II had to be used at seven times the concentration of the Green-II for satisfactory staining. Reagents used in combination (such as Red-I and Green-II) were mixed prior to application to the tissue.

Lymphoid tissue prints were obtained by touching microscope slides to the freshly cut surface of lymph node or splenic tissue obtained at surgery or autopsy. None of the specimens used came from patients with myeloma or macroglobulinemia. Stained specimens were examined under a Leitz ultraviolet microscope with a Corning #5840 exciting filter and K2, 23A, and 57A eyepiece filters.

Cells showing red, green, or mixed fluorescence under the K2 filter were differentiated according to the method of Cebra and Goldstein (*10*) in which Kodak gelatin barrier filters allow passage of only green light (57A) or red light (23A). From 100 to 300 cells were counted for each preparation and the relative percentages of each kind of staining were calculated.

The results of the experiments are summarized in Table 1. Fluorescent cells of preparations stained with both Red-I and Green-II (experiment 1) were either red or green but were rarely doubly stained. This clear and striking distinction of cells appears to indicate that, at a given time, a cell does not produce both kinds of L-chains. In all the experiments, the type I cells were more numerous, appearing approximately twice as frequently as those synthesizing type II chains. This is in accord with the relative concentrations of 7*S* γ-globulin molecules of types I and II found in normal serum.

Preparations stained with Green-Cr and either Red-I or Red-II (experiments 2 and 3) showed a significant percentage of doubly stained cells. This is interpreted to mean that single cells may produce at the same time both H-chains and L-chains of the type revealed by the appropriate stain. The green-staining cells in these experiments apparently contain 7*S* γ-globulin H-chains, either alone or together with L-chains of the other antigenic type. That the cells stain red in these experiments can indicate production of L-chains either alone or with H-chains of β_{2A}- or β_{2M}-globulins. The ratio of blended cells to green cells was generally in accord with the previously noted 2:1 predominance of type I cells.

The results of experiment 4 (Red-I, Red-II, Green-Cr) indicate the frequency of those cells staining for L-chain but not for H-chain of 7*S* γ-globulin (red-staining cells) and of those cells staining for H-chains but not L-chains of type I or type II (green-staining cells). Only a small percentage of cells stained green. Possibly the red-staining cells are producing β_{2A}- or β_{2M}-globulin. If so, such synthesis of these other immunoglobulins separate from 7*S* γ-globulin would be consistent with data of Mellors and Korngold (*11*), Burtin and Buffe (*12*), and the observations of Chiappino and Pernis on splenic cells (*13*). However, in this last investigation, some cells of the lymph node germinal center were found to contain both β_{2M}- and 7*S* γ-globulins.

Experiment 5 was performed as an internal control of the method. Preparations stained with Red-II and Green-II contained almost exclusively blended cells.

The results appear to indicate that spleen and lymph node cells may produce both L- and H-chains of 7*S* γ-globulin simultaneously but that they rarely, if at all, produce both kinds of L-chains. The ratio of type I to type II cells (2:1) is in accord with the relative amounts of these types of immunoglobulins in normal serum and also with the relative incidence of these antigenic types of myeloma globulins appearing in patients with multiple myeloma (*14*).

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References and Notes

1. G. M. Edelman and M. D. Poulik, *J. Exptl. Med.* **113**, 861 (1961); J. B. Fleischman, R. R. Porter, E. M. Press, *Biochem. J.* **88**, 220 (1963).
2. G. M. Edelman and J. A. Gally, *J. Exptl. Med.* **116**, 207 (1962).
3. J. L. Fahey, *J. Immunol.* **91**, 438 (1963).
4. M. Mannik and H. G. Kunkel, *J. Exptl. Med.* **117**, 213 (1963).
5. L. Korngold, *Ann. N.Y. Acad. Sci.* **94**, 110 (1961).
6. A. H. Coons, E. H. Leduc, J. M. Connolly, *J. Exptl. Med.* **102**, 49 (1955).
7. G. Goldstein, I. S. Slizys, M. W. Chase, *ibid.* **114**, 89 (1961); G. Goldstein, B. H. Spalding, W. B. Hunt, Jr., *Proc. Soc. Exptl. Biol. Med.* **111**, 416 (1962).
8. G. M. Bernier and F. W. Putnam, *Biochim. Biophys. Acta* **86**, 295 (1964).
9. E. C. Franklin, M. Meltzer, F. Guggenheim, J. Lowenstein, *Federation Proc.* **22**, 264 (1963); E. F. Osserman and K. Takatsuki, *Medicine* **42**, 357 (1963).
10. J. J. Cebra and G. Goldstein, in preparation.
11. R. C. Mellors and L. Korngold, *J. Exptl. Med.* **118**, 387 (1963).
12. P. Burtin and D. Buffe, *Proc. Soc. Exptl. Biol. Med.* **114**, 171 (1963).
13. G. Chiappino and B. Pernis, *Pathol. Microbiol.* **27**, 8 (1964).
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Cutaneous Sensitivity after Prolonged Visual Deprivation

Abstract. *Subjects who were placed in darkness for a week but who were otherwise exposed to a normal and varied sensory environment showed an increase in tactual acuity and in sensitivity to heat and pain. This cutaneous supersensitivity was still present several days after the termination of visual deprivation.*

Exposure of human subjects to prolonged periods of sensory and perceptual deprivation can result in a variety of behavorial and physiological changes (*1*). One of the most perplexing of these is an increase in pain sensitivity (*2*) and in tactual acuity as measured by both a two-point (smallest distance separating two points of sensitivity) threshold (*3*) and a tactual fusion method (*4*). This increase in cutaneous sensitivity, which represents the only clear instance of supersensitivity following sensory isolation, is quite pronounced. Furthermore, it seems to occur in all, or almost all, experimental subjects. The purpose of this study was to demonstrate that an overall reduction in the level of visual, auditory, tactual-proprioceptive, and social stimulation is not essential for the appearance of this phenomenon. It can occur after visual deprivation alone.

Sixteen male university students, each wearing a black mask, were placed in groups of two in an ordinary room for a period of 1 week. Apart from the exposure to constant darkness, their environment was quite normal. No gloves were worn and no restrictions were placed on their motor activity or on conversation with one another or with the experimenters. Furthermore, a radio was available in the room at all times. It was frequently in use. There were no failures; all 16 subjects successfully endured the week of darkness.

Measures of tactual acuity were taken from the palm, index finger, and forearm before and immediately after