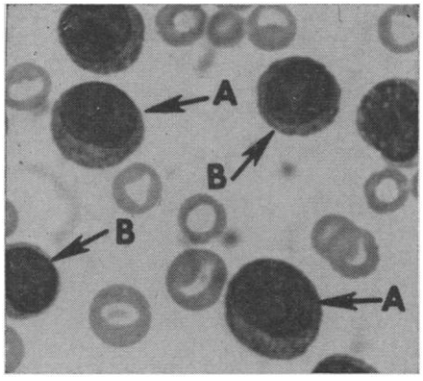

Fig. 3. Lymphoblasts (*A*) and prolymphocytes (*B*) in peripheral blood of a leukemic owl monkey. Wrights stain; × 500.

keys by *H. saimiri* and demonstrates, for the first time, development of lymphocytic leukemia in this species in association with malignant lymphoma. Further, malignant lymphoma was induced by virus given subcutaneously and intravenously, as two animals (Nos. 621-69 and 632-69) died prior to reinoculation. The intradermal route was also probably effective; animal No. 636-69 died with malignant lymphoma 1 week after the intramuscular reinoculation, and it seemed unlikely that the disease developed in 1 week. It appeared unlikely, also, that immunity developed in the six animals surviving the first inoculation, as four died of malignant lymphoma after reinoculation. Two (Nos. 622-69 and 636-69) no doubt had the disease prior to reinoculation, because one was then leukemic, and both died 1 week after reinoculation. The other two did not die of malignant lymphoma until 6 weeks (No. 638-69) and 13 weeks (No. 639-69) after reinoculation.

The incidence of malignant lymphoma in this study was 50 percent as compared with 100 percent of all owl monkeys inoculated with *H. saimiri* in earlier studies (*2–4*). The course of the disease extended to 36 weeks in the present study, whereas in previous studies no animal survived longer than 4 weeks, even when the viral inoculum was 100 times more dilute. It is difficult (if not impossible) to explain the lower incidence and longer course despite the use of an undiluted viral inoculum. Variation in virus virulence must be considered, but there has been no in vitro evidence of it thus far. A more likely explanation is variation in the host, since, with few exceptions, New World monkeys available for research are poorly defined. Genetic variability, age, indigenous viruses, parasitic diseases, and other factors might influence the pathogenicity of *H. saimiri.*

Six animals, four dying prior to reinoculation and two killed 13 weeks after the second injection of *H. saimiri,* did not have lesions indicative of malignant lymphoma. Lymphocytic hyperplasia of lymph nodes and increase in lymphocytes in hepatic sinusoids were observed in three animals (Nos. 630-69, 633-69, and 629-69), but a possible relation of these changes to virus inoculation can only be speculative. The cause of death of the four animals was not ascertained. If related to *H. saimiri,* the result would suggest that the virus can induce a disease other than malignant lymphoma. However, lack of evidence of disease suggests that infection was not established. Development of leukemia in owl monkeys inoculated with *H. saimiri* and extension of the course of disease to 70 days are of particular significance and importance as an indication of possible usefulness of the lymphoma as a model for studying chemotherapeutic procedures. Further, this leukemia induced by a herpesvirus in nonhuman primates may provide a convenient virus-host system for study of similar conditions of man.

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5. We thank Douglas Jackman, Jill Cadwallader, Martha Williamson, Martha Elliot, John Martino, and Marjorie Vallee for their technical assistance. Supported by PHS grant No. RR 00168-09.

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Immunoglobulin M and Secretory Immunoglobulin A: Presence of a Common Polypeptide Chain Different from Light Chains

Abstract. *Unique polypeptide chains have been isolated from* S-*sulfonated light-chain fractions of human serum immunoglobulin M and colostral immunoglobulin A. Their electrophoretic mobilities, molecular weights, peptide maps, amino acid compositions, and antigenic determinants are very similar or perhaps identical but differ from those of light chains and secretory piece.*

External secretions of man contain immunoglobulin A (IgA) which, in contrast to serum IgA, has a higher molecular weight and is associated with another polypeptide chain. The polypeptide, termed the secretory piece (SP), has also been found free in secretions. These considerations have been reviewed by Tomasi and Bienenstock (*1*).

After disruption of disulfide bonds, the secretory IgA (S-IgA) molecule dissociates into heavy (H) and light (L)

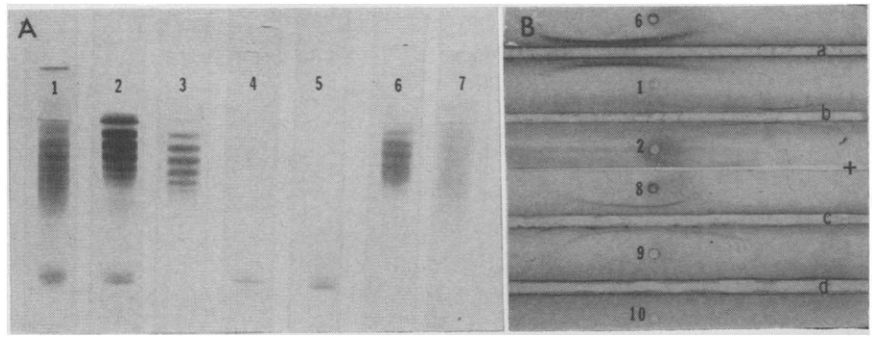

Fig. 1. Disc-electrophoretic (A) and immunoelectrophoretic (B) patterns of S-IgA and IgM subunits. (1) L-chain fraction of S-IgA; (2) L-chain fraction of IgM; (3) L-chain fraction of IgM with J chain removed; (4) J chain from S-IgA; (5) J chain from IgM; (6) L-chain fraction of IgG; (7) L-chain fraction of serum IgA (7*S*); (8) whole colostrum diluted 1 : 2; (9) SP-enriched fraction of colostrum; (10) L-chain fraction of S-Iga with J chain removed. Antiserums: (a) to L chain; (b) to J chain; (c) to SP.

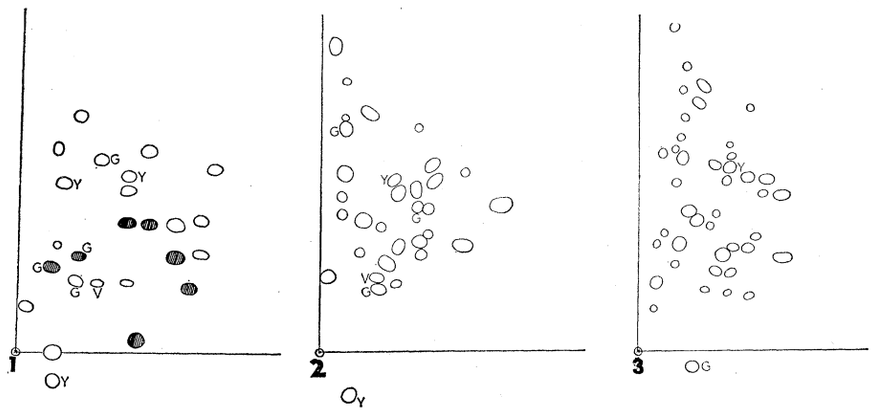

Fig. 2. Peptide maps of S-IgA and IgM subunits. (1) J chain from IgM. The peptide map of J chain from S-IgA was identical. Shaded: Histidine- and tyrosine-containing peptides detected by use of Pauly's reagent; (2) L-chain fraction of IgM without J chain (κ type, Waldenström's macroglobulinemia); (3) L-chain fraction of S-IgA without J chain. The main peptides of the L-chain fraction of IgG were identical. G, V, Y: colors gray, violet, and yellow, after ninhydrin staining.

chains, as well as a fast-moving protein detectable in the L-chain fraction by alkaline electrophoresis in urea. The fast-moving protein has been found in human (*2*) and rabbit (*3*) colostrum IgA; it was assumed that this material was SP (*3*). Evidence has been presented recently that rabbit colostrum IgA contains a unique polypeptide chain, called J, in addition to SP (*4*).

While examining immunoglobulin M (IgM) that had been isolated from serums of patients with Waldenström's macroglobulinemia, we observed in the L-chain fraction a protein identical in electrophoretic mobility with the fast-moving protein from S-IgA (Fig. 1A). We studied the occurrence, composition, and immunochemistry of this fast-moving protein. We refer to it as J chain on the basis of its analogy with the fast-moving polypeptide from rabbit S-IgA (*4*).

Human S-IgA, serum IgG, IgA (7*S*), and Waldenström's IgM were purified (*5*) and subjected to oxidative sulfitolysis (*6*), with subsequent separation of H and L chains on Sephadex G-200 in 5*M* guanidine hydrochloride. When L-chain fractions were examined by polyacrylamide disc electrophoresis in 10*M* urea, *p*H 9.4 (*7*), J chains were observed in those from S-IgA and IgM but not in fractions of the other two serum immunoglobulins.

To isolate J, the L-chain fractions of S-IgA and IgM were chromatographed on diethylaminoethyl (DEAE)-Sephadex with 8*M* urea (*8*). Two peaks were observed; the first, larger fraction contained immunochemically and electrophoretically pure L chains; the second, smaller fraction contained the J chain. Disc electrophoresis (*7*) of highly concentrated J chains demonstrated no detectable contamination by L chains. The yield was 8 and 4 mg from 100 mg of L chains of S-IgA and IgM, respectively.

The approximate molecular weight of the J chain was suggested by its elution with the L-chain fraction from gel filtration through Sephadex G-200. From polyacrylamide electrophoresis in sodium dodecylsulfate (*9*), the molecular weight of J chain was estimated to be 26,000.

Amino acid analysis indicated that the compositions of J chains from S-IgA and IgM were either very similar or perhaps identical but were strikingly different from those of the L chains of S-IgA or IgM (Table 1). Of particular interest was the occurrence of approximately twice as many cysteine residues per thousand residues as were found in L chains. The high content of aspartic acid is consistent with the anionic behavior of J chains on electrophoresis.

Peptide maps stained with ninhydrin or Pauly's reagent (*10*) (Fig. 2) revealed complete identity of J chains from S-IgA and serum IgM. Yet, the peptide maps of J chains were quite different from peptide maps of H chains, L chains from IgG, and L chains from S-IgA and IgM after removal of J chain by DEAE chromatography.

By immunoelectrophoresis (*11*) (Fig. 1B), J chains from S-IgA and IgM were not distinguishable; one antiserum, produced against S-IgA–derived J chain, precipitated with both antigens, and both had the same electrophoretic mobility. L chains, SP, and J chains, besides differing in electrophoretic mobility, precipitated exclusively with their homologous antisera; they did not share any common antigenic determinants.

Thus, the human J chain may be defined as a polypeptide chain of a molecular weight of about 26,000. It displays a fast electrophoretic mobility that is probably due to a relatively high proportion of acidic amino acids. A distinguishing feature is the high content of cysteine. The J chain is attached to IgM in the particular preparations we examined and to S-IgA but not to 7*S* IgG or IgA molecules. It occurs in a most probable ratio of one chain per immunoglobulin molecule and is released only after disruption of disulfide bonds (*12*).

Table 1. Amino acid compositions of J chains, L chains, and secretory piece. Analyses were performed on a Beckman 120 C amino acid analyzer. All values represent the average of two or more analyses. The calculations do not include tryptophan. Values are expressed as residues per thousand to allow comparison of the relative compositions of chains which vary in molecular weight.

Amino acid	J chains		L chains		Secretory piece*
	IgA	IgM	IgA	IgM	IgA
Aspartic	150.8	160.5	84.0	81.0	63.0
Threonine	77.7	87.6	83.3	89.6†	75.5
Serine	47.5	52.5	111.7	122.6†	100.8
Glutamic	113.9	109.2	111.6	118.7	61.3
Proline	73.0	71.7	73.5	56.7	64.8
Glycine	22.0	21.0	67.4	66.3	254.0
Alanine	48.9	47.7	70.3	77.2	76.5
Cysteine‡	51.2	50.6	26.9	25.3	22.2
Valine	76.4	79.6	76.7	70.1	66.4
Methionine	9.7	7.2	6.0	5.2	10.2
Isoleucine	68.0	65.2	36.6	33.4	17.7
Leucine	68.6	64.2	79.2	79.6	70.0
Tyrosine	46.5	44.8	42.2	37.2	23.4
Phenylalanine	11.9	9.8	30.2	38.1	30.5
Lysine	48.6	41.3	56.7	46.3	29.8
Histidine	10.3	8.3	14.9	11.0	9.7
Arginine	74.9	78.7	28.7	41.5	24.4

* Recalculated from the data of Tomasi and Bienenstock (*1*). † Values corrected for losses due to acid hydrolysis. ‡ Calculated as cysteic acid after performic acid oxidation (*14*).

An analogy with the rabbit T chain (*3*) and J chain (*4*) is suggested by the criteria of electrophoretic mobility, molecular weight, and occurrence in S-IgA; but other characteristics were not determined.

The properties of J chain do not match those of human SP. The latter has a molecular weight of at least 76,000 either free in colostrum or isolated from S-IgA (*13*) and displays amino acid composition (Table 1), electrophoretic mobility, and antigenic determinants which differ distinctly from those of the J chain (Fig. 1B). If SP were composed of two or more J chains, the relative proportions of amino acids should agree. Also, increasingly severe conditions for splitting and dissociation should increase the yield of J chains at the expense of SP. This we did not observe (*12*); SP was not present in IgM.

Since J and L chains resemble each other in ranges of molecular weights only, there are no grounds for assuming that they are related.

The apparent identity of J chains from S-IgA and IgM is interesting in view of their presence in two classes of immunoglobulins, in two body fluids, and in two states of health. The attribute unifying the two immunoglobulins is their polymeric configuration. It may be permissible to speculate that the J chain with its unusually high cysteine content may aid in maintaining the tertiary structure of the polymeric immunoglobulin molecule.

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11. Antiserum to SP was prepared by immunosorption of antiserum to human colostral IgA onto human serum globulins attached to Sepharose 4B [J. Mestecky, F. W. Kraus, and S. A. Voight, *Immunology* **18**, 237 (1970)]. Antiserum to L chain was obtained in a similar manner by attaching human IgG to the column and desorbing the antibodies with glycine hydrochloride. Antiserum to J chain was produced in rabbits by repeated multiportal injection of homogenized polyacrylamide-gel slices containing J chains in complete Freund's adjuvant.
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15. We thank Dr. M. E. Koshland for a stimulating discussion. After completion of this manuscript, we received from her a preprint (*Nature*, in press) in which Drs. M. S. Halpern and M. E. Koshland describe the occurrence of J chain in 11*S* serum IgA from human myeloma. We thank Dr. F. W. Kraus for his contributions; Mrs. Rose Kulhavy and Miss Freda T. Moore for technical assistance; Dr. J. C. Bennett for continual encouragement; and Dr. Alexander Lawton for criticism of the manuscript. Supported by PHS DE-02670 and the John Hartford Foundation grants.

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Stimulation in vitro with Protein Carrier of Antibodies against a Hapten

Abstract. *Rabbits were immunized both with lysozyme and with dinitrophenylated bovine serum albumin. No antibodies against the dinitrophenyl hapten were produced when cell suspensions of their spleens were exposed in vitro to dinitrophenyl-ovalbumin, whereas a positive response was obtained with dinitrophenyl-lysozyme. Moreover, when spleen cells from rabbits previously immunized with dinitrophenyl–bovine serum albumin were exposed in vitro to bovine serum albumin alone, they produced antibodies against the dinitrophenyl hapten.*

In studies of haptenated proteins no secondary response can be obtained with a conjugate of the hapten to an unrelated protein (*1, 2*). A secondary response to the hapten may be obtained when mixtures of cells derived from animals immunized with the initial haptenated protein and other animals immunized with a second protein alone were used (*1, 3*). Moreover, preliminary immunization with the hapten conjugate of one protein, followed by a sec-

Table 1. The effect of different protein carriers on the anamnestic immune response toward the dinitrophenyl (DNP) group. The immune response was tested by [^{14}C]thymidine incorporation and by inactivation of dinitrophenylated bacteriophage T4. Spleen cell suspensions from individual rabbits were cultured on day 0 in the absence (control) or presence of antigen whose concentration varied from 1 to 100 μg per culture tube. After 24 hours the cells were exhaustively washed and then resuspended in M 199 medium supplemented with 20 percent normal rabbit serum. On days 2, 4, 6, and 8 the medium of cell cultures was changed.

Group	Rabbits immunized with	Experiments (No.)	Spleen cells exposed in vitro to DNP conjugated with			
			BSA	Ovalbumin	Lysozyme	BSA
1	DNP-BSA	5	+*	−	−	+
2	Lysozyme DNP-BSA	2	+	−	+	+
3	DNP-BSA lysozyme	5	+	−	+	+

* Positive (+) and negative (−) symbols represent the presence and the absence of a significant (at least tenfold) difference between antigen-stimulated culture and nonstimulated cultures, in terms of both the rate of DNA synthesis (as measured on day 2) and titer of antibodies against the dinitrophenyl hapten (as measured on days 6 and 8).