ALLOTYPIC MARKERS ON RABBIT IgA*

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An immunoglobulin corresponding to human IgA in electrophoretic mobility was identified in rabbit colostrum and shown to bear the allotypic markers of both genetic loci *a* and *b*† by Feinstein (1963). The data reported here show that the molecules bearing these markers constitute a major portion of the population of IgA molecules.

The interrelationship of the allotypic markers in IgA and IgG parallels that observed by Todd (1963) for IgG and IgM. Since the light chains are believed to be common to each of the major classes of immunoglobulin, one might expect to find the locus *b* markers characterizing these chains on each of these immunoglobulins. The markers of locus *a* are on the Fd fragment of the H chains (Stemke, 1964). Evidence that the allotypic markers on IgM

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†The allotypic specificities a1,a2, and a3 of locus *a* (Dray *et al*, 1962) are located on the heavy (H) chain and the specificities b4,b5,b6, and b9 (Dubiski and Muller, 1967) of locus *b* are located on the light (L) chain (Stemke, 1964). The immunoglobulin molecules and fragments are named following the system recommended for human immunoglobulins by a committee of the World Health Organization (Ceppellini *et al*, 1964).

are present on substantially all of the IgM molecules has been provided by Todd and Inman (1967). Since each of these immunoglobulin classes are characterized by the presence of distinct H chains, it is surprising that these markers should be similarly shared. This phenomenon is all the more difficult to rationalize in the light of recent evidence (Inman, 1967; Koshland, 1967, and Porter, 1967) that these allotypic specificities are determined by the primary amino acid sequence.

The initial work of Feinstein was confirmed and extended by Sell (1966) who isolated the IgA immunoglobulin and confirmed the presence of allotypic markers of both genetic loci. Cebra and Robbins (1966) confirmed the presence of the allotypic markers of locus *b* but were not able to obtain evidence for those of locus *a*. Lichter (1967) has presented evidence that the detection of these specificities may be a function of pH. As pointed out by Kelus (1967), it would be desirable to have quantitative information on the extent to which allotypic markers of locus *a* are present on IgA. Such information is presented in this communication.

MATERIALS AND METHODS

IgA was isolated from the milk of an a1,a3,b5 rabbit* obtained 15 days after kindling following the method of Cebra and Robbins (1967). It was shown by Ouchterlony diffusion to react with an absorbed anti-IgA serum+ and to be free of material reacting with an absorbed serum specifically directed against the Fc portion of IgG. The $S_{20,w}$ corrected to infinite dilution was found to be 11.3 in agreement with the value of 10.8 reported by Cebra and

*The rabbit also possessed the A-11 agglutinating specificity recently described by Mandy and Todd (1968).

+A gift from Prof. Pernis.

Robbins (1967). The material thus appears to be IgA combined with its transport piece (Tomasi *et al*, 1965).

The material was iodinated using I^{125} according to the method of McFarlane (1958) to obtain material giving 6 x 10^5 cpm per mg. Aliquots (0.1 ml) of the test solution were counted after addition to a mixture comprising 6 ml absolute ethanol, and 8 ml of toluene containing 8 gm PPO and 100 mg POPOP per liter in a liquid scintillation vial filled with Cab-O-Sil.

The anti-allotype sera were prepared by injection of anti-ovalbumin specific precipitate bearing the allotypic determinant into a rabbit lacking this determinant following the method of Oudin (1960). The anti-Fcγ serum was prepared by absorbing sheep antiserum directed against IgG (obtained by ammonium sulfate precipitation and DEAE chromatography) with Fab´ obtained by pepsin hydrolysis of IgG (Nisonoff *et al*, 1960). This removes any antibody to common antigenic determinants which may be present on the Fd regions of the H-chains (Todd, Walz, and Osterland, 1967). The anti-IgA serum was absorbed with IgG to render it specific for IgA.

RESULTS

The ability of our anti-allotype sera to precipitate the radioiodinated IgA was determined as a function of pH, and pH 7 was found to be optimal. Lichter (1967) found the pH of the reaction medium to be critical for precipitate formation between IgA and antisera for the group *a* allotypic markers, pH 5 being optimal. Presumably the pH requirement is a function of the particular antiserum or IgA sample used since neither Feinstein nor Sell found it necessary to use this low pH. Accordingly, precipitations were carried out in a 0.1 molar phosphate buffered saline at pH 7. Starting with samples containing 20,000 cpm of radioiodinated

IgA, one-fifth aliquots of the supernatants from precipitations with anti-allotype sera were counted. The results obtained are summarized in Table I.

TABLE I

Precipitation of radioiodinated immunoglobulin

Precipitating Serum	$IgA \cdot I^{125}$ percent			$IgG \cdot I^{125}$ percent		
	1st	2nd	total	1st	2nd	total
Anti-a1	46	6	52	24	3	27
Anti-a3	36	12	48	9	1	10
Anti-b5	80	5	85	73	10	83
Anti-Fcγ	<1		<1	90		90
Anti-IgA	76	11	87	<1		<1

Trichloracetic acid (10%) precipitated 97% of the IgA counts and 92% of the IgG counts.

It was found that 46%, 36% and 80% of the counts were precipitated by sera directed against allotypes a1, a3, and b5 respectively in the initial precipitation. A second precipitation after addition of unlabelled IgA to the supernatant (Dray and Nisonoff, 1963) brought the total counts precipitated to 52%, 48% and 85% respectively. From this it is apparent that a substantial fraction of the molecules bear each of the allotypic determinants.

Also included in Table I is a summary of similar results obtained with IgG isolated by ammonium sulfate precipitation and DEAE chromatography from the same rabbit. The ability of the absorbed anti-IgA serum to precipitate the counts from radioiodinated IgA and its inability to precipitate the counts from the radioiodinated IgG demonstrate that the carrier molecules in each case are different. This result is confirmed by the converse ability of the anti-Fcγ to precipitate the counts of the radio-

iodinated IgG, and its inability to precipitate the counts of radioiodinated IgA.

Cebra and Small (1967) have shown the fingerprint of H chain isolated from colostral IgA to differ markedly from the fingerprint of H chain from IgG. The material used in our study was isolated from rabbit milk obtained 15 days after parturition. To demonstrate that the transport piece was not hiding antigenic sites which would otherwise react with anti-Fcγ, IgA isolated from the milk was radioiodinated and dissociated to its 7S units in 5 M guanidine hydrochloride and passed through a Sephadex G200 column as described by Hong *et al* (1966). The material eluted in the main peak after dialysis against 0.1 M pH7 phosphate buffered saline was 90% precipitated by the anti-IgA and only 8% by the anti-Fcγ.

DISCUSSION

As expected, the locus *b* allotypic determinants are present on IgA to approximately the same extent as on IgG. The finding that nearly all of the molecules of IgA carry the allotypic determinants of locus *a* demonstrates clearly that this cross-reactivity cannot be attributed to minor contamination with IgG. Studies are in progress to compare the nature of the allotypic determinants on IgA with those on IgG as reported for IgG and IgM (Todd and Inman, 1967).

It will be noted that the counts precipitated of allotypes a1 and a3 total 100%. Thus, it may be that all of the IgA molecules carry one or the other of the two allelic allotypes in a heterozygotic animal. Essentially all of the IgM molecules carry the H chain allotypic determinants in homozygotic rabbits (Todd and Inman, 1967). On the other hand, if the secretory IgA in

rabbit milk is assembled in the mammary gland from locally synthesized transport piece and from 7S IgA synthesized elsewhere (Asofsky and Small, 1967), one might expect to find two allelic allotypes mixed in a single IgA-transport piece complex. In this case, the fact that the percentages of the two locus *a* allotypes precipitated total 100% does not necessarily mean that all IgA monomer units in the transport piece complex carry allotypic determinants. Under the conditions used here, certain molecules would be counted under both allotypes a1 and a3. This possibility is being investigated. Further discussion of the genetic implication of the sharing of genetic markers by the classes of rabbit immunoglobulins has been presented elsewhere (Todd and Inman, 1967)

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REFERENCES

Asofsky, R. and Small, P.A., Science, 158, 932 (1967).
Cebra, J.J. and Robbins, J.B., J. Immunol. 97, 12 (1966).
Cebra, J.J. and Small, P.A., Biochemistry, 6, 503 (1967).
Ceppellini *et al.*, Bull. Wld. Hlth. Org., 30, 447 (1964).
Dray, S., Dubiski, S., Kelus, A., Lennox, E. and Oudin, J., Nature, Lond., 195, 785 (1962).
Dray, S. and Nisonoff, A., Proc. Soc. exp. Biol. Med., 113,20 (1963
Dubiski, S. and Muller, P.J., Nature, Lond., 214, 696 (1967).
Feinstein, A., Nature, Lond. 199, 1197 (1963).
Hong, R., Pollara, B. and Good, R.A., Proc. nat. Acad. Sci., 56, 602 (1966).
Inman, J.K., Fed. Proc., 26, 479 (1967).
Kelus, A.S., Nobel Symposium, 3, 329 (1967).
Koshland, M.E., Cold Spr. Harb. Symp. quant. Biol., 32, (1967), in press.
Lichter, E.A., J. Immunol., 98, 139 (1967).
Mandy, W.J. and Todd, C.W., Vox Sang., 14, (1968), in press.
McFarlane, A.S., Nature, Lond., 182, 53 (1958).
Nisonoff, A., Wissler, F.C., Lipman, L.N. and Woernley, D.L., Arch. Biochem. and Biophys., 89, 230 (1960).
Oudin, J., J. exp. Med., 112, 107 (1960).
Porter, R.R., Biochem. J., 105, 417 (1967).
Sell, S., Immunochem., 4, 49 (1967).

Stemke, G.W., Science, 145, 403 (1964).
Tomasi, T.B., Tan, E.M., Solomon, A. and Prendergast, R.A., J. exp. Med. 121, 101 (1965).
Todd, C.W., Biochem. Biophys. Res. Comm., 11, 170 (1963).
Todd, C.W. and Inman, F.P., Immunochem., 4, 407 (1967).
Todd, C.W., Walz, M.A. and Osterland, C.K., Proc. Soc. exp. Biol. Med., 126, 383 (1967).