NONALLELIC BEHAVIOR OF THE OZ GROUPS IN HUMAN LAMBDA IMMUNOGLOBULIN CHAINS

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Communicated by James A. Shannon, April 19, 1968

Recent amino acid sequence studies of immunoglobulin polypeptide chains have been clarifying the structural basis for serologically defined allelic forms, classes, and subclasses of immunoglobulins.

We recently described two forms of the lambda light polypeptide chains of human immunoglobulins which were recognized by a rabbit antiserum and were designated Oz (+) and Oz (−).[1] These subtypes correlated with a single amino acid difference at position 190 in the common region of lambda Bence Jones proteins.[2] Oz (+) proteins have lysine and Oz (−) proteins have arginine at that position. Further studies were undertaken to determine if these were allotypic variants of human lambda chains comparable to the Inv groups of kappa chains. Chemical identification of the Oz peptides was done in an unselected group of normal individuals, and the probability that the Oz groups are allelic was calculated. This study shows that, unlike the Inv groups of kappa chains, the Oz groups do not behave as alleles.

Materials and Methods.—Immunoglobulin G (IgG) was purified from normal human serum by *O*-(diethylaminoethyl) cellulose (DEAE-cellulose) chromatography on Whatman DE52, using a buffer of 0.05 *M* tris(hydroxymethyl)aminomethane-HCl at pH 8.0. Light and heavy chains were separated by reduction of IgG with 0.1 *M* dithioerythritol or 0.25 *M* 2-mercaptoethanol followed by Sephadex G-100 gel-filtration in 1 *N* acetic acid.

Light chains were dissolved in 0.5% NH_4HCO_3 and denatured by placing in boiling water for 30 sec. They were then digested with trypsin (Worthington, L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone-treated), using an enzyme-substrate ratio of 1:100 at 37° overnight.

Tryptic digests were spotted on Whatman 3MM paper of 1.2-m length and subjected to high-voltage electrophoresis at pH 3.6 and 4,600 v for 4.5 hr.

Papers were stained with the phenanthrenquinone reagent for arginine[3] followed by acid ninhydrin. Controls for each electrophoretic run consisted of an arginine-lysine standard as well as tryptic digests of Oz (+) and Oz (−) lambda Bence Jones proteins and a kappa Bence Jones protein.

In one case, the peptides were eluted with H_2O, hydrolyzed for 24 hr with 6 *N* HCl at 108°C, and amino acid analysis performed both by the method of Dreyer and Bynum[4] on paper and the Spinco model 120C amino acid analyzer.

Electrophoresis of the tryptic digests of Oz (+) and Oz (−) lambda Bence Jones protein and a kappa Bence Jones protein showed clear separation of the Oz (+) and the Oz (−) peptides and a histidyl-lysine dipeptide deriving from the common region of kappa light chains. No other common or variable region peptides have a comparable positive charge at pH 3.6 (Fig. 1).

Results.—Both Oz (+) and Oz (−) peptides were identified in light chains from ten consecutive unselected and genetically unrelated normal persons (Fig. 1). The Oz peptides were isolated from the light chains of one individual and subjected to amino acid analysis on paper. Although slight contaminants were present, the major amino acids were clearly serine, histidine, arginine for one peptide

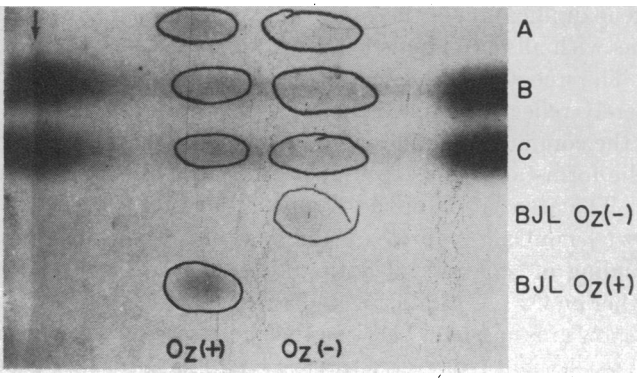

FIG. 1.—High-voltage electrophoresis of tryptic digests of light chains. *A*, *B*, and *C* are digests of light chains from three different normal individuals. Digests of Oz (+) and Oz (−) lambda Bence Jones proteins are also shown. The arrow indicates a His-Lys dipeptide from the common region of kappa light chains.

and serine, histidine, lysine for the other. Quantitative amino acid analysis of these peptides showed the ratio of histidine Oz (+) to histidine Oz (−) to be 1:2.5. This ratio gives a rough estimate of the proportion of Oz (+) and Oz (−) lambda polypeptide chains in this individual.

Calculation of the probability that the two Oz groups represent allelic genes was done by the Hardy-Weinberg formula and by the chi-square method. Both methods gave essentially identical results. If the Oz groups were alleles of equal frequency, the probability of finding ten consecutive heterozygotes by chance is $(0.5)^{10}$, or less than 1 in 1000. (In this situation, the maximum frequency of heterozygotes would be 0.5, occurring with $p = q = 0.5$ where p = Oz (+) frequency and the Oz (−) frequency $q = 1 - p$.)

Lambda Bence Jones proteins have one or the other Oz groups, thus their frequency in Bence Jones proteins might reflect the frequency of hypothetical alleles. We have tested, by chemical means, a total of 40 lambda Bence Jones proteins and find 60 per cent Oz (−) and 40 per cent Oz (+). Quatrocchi *et al.*,[5] in a study of 40 proteins, found 87.5 per cent Oz (−), and Tischendorf and Osserman[6] found 83 per cent Oz (−). Milstein[7] and Putnam[8] have published data for an additional nine proteins which include the amino acid at position 190. If data for all these studies are combined, 25 per cent of 107 proteins are Oz (−), while 75 per cent are Oz (+). If the Oz groups are assumed to be alleles, the gene frequency of the Oz (−) group would be 0.75 from the Bence Jones protein data, and the probability of finding ten consecutive heterozygous individuals is diminished to $(0.375)^{10}$ or about 1 in 20,000. It is unlikely that the Oz groups are allelic.

Discussion.—Prior to this study, the finding of Oz (+) and Oz (−) peptides, i.e., either Arg or Lys at position 190, in separate Bence Jones proteins seemed very analogous to the allelic Inv groups of kappa chains (valine or leucine at position 191). They were considered to be another possible example of allelic forms of human immunoglobulins. The present study, however, strongly suggests that the Oz groups do not reflect allelic genes.

Two genetic mechanisms could account for the nonallelic behavior of the Oz

groups. A gene duplication of the common region of lamdba chains would result in two cistrons with all individuals homozygous for both the Oz (+) and the Oz (−) forms. The unequal frequencies of Oz (+) and Oz (−) Bence Jones proteins may merely reflect differences in the expression of the genes. Other rare variations in the common region of lambda chains, such as reported by Milstein[7] could be allelic forms analogous to that of the βS chain and normal β chains of hemoglobin. The association of these unusual lambda common region variants with either Oz (+) or Oz (−) forms of lambda chains would suggest gene duplication. The finding of separate allelic markers, one present on Oz (+) lambda chains, the other on Oz (−) chains, would permit family studies to be performed. These might show crossing-over between the allelic markers that would also argue for separate Oz (+) and Oz (−) cistrons. Other proof of the gene duplication hypothesis would come from the finding of a lambda Bence Jones protein containing both Oz (+) and Oz (−) peptides in a single unique sequence—the result of unequal crossing-over, similar to Hgb lepore.[9]

Alternatively, ambiguity in translation could result in the insertion of either arginine or lysine at position 190 of lambda chains during lambda-chain biosynthesis. Three possible ways in which a translational error could occur are: (*a*) ambiguity of a messenger RNA (mRNA) codon so that either an arginine or lysine transfer RNA (tRNA) could recognize this codon; (*b*) the existence of a tRNA for either amino acid which can recognize a codon for both amino acids and which is used only for position 190 of lambda chains; or (*c*) an unusual activating enzyme which acylates a tRNA with either arginine or lysine. Translational errors have been proposed by Popp[10] to account for a possible ambiguous site in the α chain of BALB/c hemoglobin, by von Ehrenstein[11] for amino acid duplicities in rabbit hemoglobin α and by Potter *et al.*[12] to account for variability in the N-terminal region of immunoglobulin polypeptide chains. There is no data at present which would favor either the gene duplication or translational error hypothesis in human lambda light chains. However, if there are two genes for lambda-common regions, the gene duplication might be of recent origin because of the relative conservation of the lambda-common region in man.

Questions about the genetic mechanisms and evolution of the Oz groups and their distribution in various genetically isolated populations are of interest in understanding this new example of immunoglobulin heterogeneity and are currently under investigation.

Summary.—Two forms of human lambda immunoglobulin light chain with either lysine (Oz +) or arginine (Oz −) at position 190 were identified in normal human serum immunoglobulins. Light chains from each of ten randomly selected normal individuals had both Oz (+) and Oz (−) peptides. The finding of both forms of all individuals strongly suggests that the Oz groups do not behave as alleles ($p < 0.001$). A duplication of the common region gene for lambda chains or a translational ambiguity at position 190 can explain this finding.

The author wishes to thank Drs. J. L. Fahey, L. Hood, W. D. Terry, K. S. Brown, and A. Steinberg for helpful discussion and advice. He also thanks Mrs. Diane Pincus for her competent technical assistance.

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