ABSENCE OF PREFERENTIAL REASSOCIATION BETWEEN HEAVY AND LIGHT CHAINS OF TWO HUMAN IMMUNOGLOBULINS FROM COMMON CELLULAR ORIGIN.

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SUMMARY.

Competitive hybridization was performed, using monoclonal immunoglobulin chains derived from 2 human myeloma proteins produced by the same patient (Im), and having a common cellular origin. Both proteins had an identical H chain, but differed by their L chains, one being κ , the other λ . Whereas, in vivo, the ratio of the Im(κ)/Im(λ) was previously reported to be of 8/1, both the κ and the λ chains were found to hybridize in vitro with equal efficiency. This ruled out that a hysteresis phenomenon may have been the basis of the preferential reassociation usually observed for the autologous systems. This preference thus appears of genetic significance, implying that some selection process in the choice of H-L pairs must occur at the cell level.

It has been shown that isolated H_A and L_A monoclonal polypeptide chains of immunoglobulins derived from the same myeloma protein preferentially reassociated whenever autologous L_A and heterologous L_B chains were allowed to competitively hybridize for the same H_A chains (1-4). Structural basis for this preference, which occurs in 80% of the cases (3), relies exclusively on idiotypic differences, i.e. differences in the amino acid sequence of the variable regions of both the H and the L chains (2-4).

A case of myeloma disease (Im), characterized by the simultaneous double production of two proteins by the same cell has been recently reported (5,6). One protein is of the $\gamma_1\kappa$ type, whereas the other is a $\gamma_1\lambda$. From immunochemical analysis, it appears that the γ_1 chain is the same in both proteins, since they share identical idiotypic determinants (5). The serum level of the $\gamma_1\kappa$ is, however, 8 times more abundant than that of the $\gamma_1\lambda$ protein. Since this might be due either to the fact that a preferential reassociation could be observed in the $\gamma_1\kappa$ versus the $\gamma_1\lambda$ system, or to a differential rate of synthesis of κ/λ , we compared the efficiency of both light chains to compete for the same and common heavy chain.

MATERIALS AND METHODS.

Isolation of myeloma proteins Im(κ), and Im(λ), was done according to Oriol et al. (5). Heavy and light chains were separated on Sephadex G 100 columns, in 1.0 M propionic acid (7) after the myeloma proteins had been mildly reduced with 8 mM di-thio-threitol, alkylated with 20 mM iodoacetamide (3), and labeled either with [^{125}I] or [^{131} I],according to Mc Farlane (8).

Competitive hybridizations were thus realized as indicated in detail in (3). Essentially, to the unlabeled H chain was added, while still in 1.0 M propionic acid, equimolar amounts of distinctively labeled $\varkappa$ and λ chains (H/$\varkappa$/λ : 1/2/2, on a molar basis). After exhaustive dialysis versus 0.1 M Tris-HCl buffer, 0.15 M NaCl, pH 8.0, the mixture was analyzed by centrifugation in a Spinco ultracentrifuge (L2-50), using a SW 36 L rotor, run at 36,000 rpm for 27 hours, in order to separate the hybrid molecules contained in the 6.7S peak from the excess of free light chains (2.5 S). Radioactivity measurement of both peaks allowed to determine the yield of reconstituted molecules, and the relative contribution of $\varkappa$ and λ chains to the hybrid IgG (3).

NH_2-terminal analysis of isolated chains was performed by dansylation in 8 M urea (9). The typing for the variability subgroups of the $\varkappa$ and of the H chains was done according to Moulin and Fougereau (10), and to Moulin et al.(11).

RESULTS.

Chemical characterization of the isolated chains.

Both the $\varkappa$ and the λ chains had an unblocked NH_2-terminus, which were Asx and Glx, respectively. Isolation of the characteristic Cystein 23-containing peptide released upon hydrolysis with trypsin+chymotrypsin from the $\varkappa$ chain allowed to assigned it to the $V_\varkappa I$ variability subgroup (10). Heavy chains from Im($\varkappa$) and Im(λ) had both a blocked NH_2-terminus. Analysis of the Cystein-containing characteristic peptides indicated that both belonged to the $V_H I$ subgroup (11). In addition, partial finger-prints of both chains were identical, an observation that was in agreement with the identity observed for their idiotypic determinants (5).

Competitive hybridizations.

Since both H chains looked identical by immunochemical and biochemical criteria, and because of Im(λ) shortage, the competitive hybridization was set up with only the H chains derived from one protein, Im($\varkappa$). An equimolar amount of [^{125}I] and [^{131}I] was added to the heavy chain preparation (H/$\varkappa$/λ : 1/2/2, on a molar basis). The overall yield of reconstituted molecules was 55%, in agreement with usually reported values (3,4). It was observed (table 1) that both the $\varkappa$ and the λ chains equally contributed to the reconstituted molecules, as opposed to the Im($\varkappa$)/Im(λ) ratio of 8/1 found in the serum of the patient suffering this double producing myeloma tumor.

DISCUSSION.

Preferential reassociation of monoclonal H and L chains derived from the same immunoglobulin molecule relies essentially on individual structural features of variable regions of both chains (2-4). Since chains used for the reassociation are only mildly reduced, a hysteresis phenomenon must be ruled out (12,13). In a previous paper, we have reported that, whenever reassociation was performed with chains that had been completely reduced, denatured, and reoxidized, preference was not abolished, thus strongly suggesting that the hysteresis hypothesis was very unlikely (4). The

Table 1

Analysis of the hybrid molecules by ultracentrifugation in a sucrose gradient.

	c.p.m. of each labeled light chain	
	Layered.	Recovered in the 6.7S peak.
[^{125}I] κ chains	154,389 (100%)	20,842 (13.5%)
[^{131}I] λ chains	103,413 (100%)	14,685 (14.2%)
Ratio κ/λ	1	0.95

To 200 µl of cold H chains (0.25 mg/ml in 1.0 N propionic acid), were added 200 µl of [^{125}I] κ chains and 200 ul of [^{131}I] λ chains, at the same concentration, so that the ratio H/κ/λ was 1/2/2 on a molar basis. After exhaustive dialysis against a pH 8.0 buffer, the mixture was analyzed by ultracentrifugation on a sucrose gradient (5 to 15%). Taking into account the four-fold excess of light chains used for the hybridization, the overall ratio of reconstituted IgG was 55%.

opportunity provided by a biological model, in which the same cell was shown to synthetize 2 myeloma proteins having an identical heavy chain, but differing by their light chains, each pertaining to a discrete type (κ and λ), allowed to fully corroborate that the preferential reassociation was solely dependent on the primary structure and was, therefore, genetically determined, which indicates that some selection must occur at the cell level.

In the present case, since the in vitro reconstitution clearly showed that equal ability of both the κ and the λ chains to reassociate with the same H chain (associated to the κ chain in vivo), the ratio of the Im(κ)/Im(λ) molecules of 8 cannot be explained by a preference of the κ chain for the heavy chain, and would rather reflect a differential rate of synthesis of the κ and the λ chains, that would be in favor of the former. Such an unbalanced production of discrete chains in myeloma cell lines have been reported (14).

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