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Immunology

Immunoglobulin chain loss in hybridoma lines

(immunoglobulin variants/chromosome loss)

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ABSTRACT **Hybrid cells secreting one, two, or three different immunoglobulins were constructed. The loss of immunoglobulin heavy or light chain expression was monitored. Chain loss was random only in lines with an excess of active light chain genes over heavy chain genes. In all other combinations preferential heavy chain loss was observed. Variant cells altered in heavy or light chain synthesis exhibited an altered chain loss pattern. It is therefore proposed that free immunoglobulin heavy chain is toxic for the cells. The interdependence of the two gene products gives a possible molecular explanation of apparent directed chromosome loss in hybrid cells.**

If a specialized cell, such as a B lymphocyte, produces a large amount of a protein that, like immunoglobulin (Ig), is composed of two different polypeptide chains, one or the other chain will, in general, be synthesized in excess. That is, precise stoichiometry is a special case, which would require a special mechanism to achieve and which is, indeed, not achieved in most cases. B lymphocytes, for example, produce an excess of light (L) chains over heavy (H) chains. Natural selection, during ontogeny as well as during phylogeny, will ensure that the free form of the chain made in excess will not be toxic for the cell synthesizing it. But there will be little or no comparable selective pressure against the free form of the one not produced in excess. Therefore, we would expect that experimentally reversing the ratio of chain syntheses would prove to be detrimental or even lethal to the cell.

The Ig system of the mouse is particularly suitable for studying this possibility. Ig is secreted in large quantities by myeloma and hybridoma lines. The H and L chains of different Igs can easily be discriminated on $NaDodSO_4$/polyacrylamide gels. In Ig-secreting mouse–mouse hybridoma cells (similar to those used in this study) H or L chain loss was correlated with the loss of one copy of chromosome 12 or 6, respectively. Only one of the two homologous chromosomes directs Ig synthesis (1). Hence, chain loss due to chromosome loss will be an all or none phenomenon easy to detect. Early studies on myeloma cells indicated that loss of H chain expression was a frequent event that preceded the loss of L chain expression (2). This observation is compatible with the idea that free H chain is toxic for the cell. The present study reinforces this notion.

MATERIAL AND METHODS

Cell Lines and Culture Conditions. Cells were grown in Dulbecco's modified Eagle's medium (GIBCO) supplemented to contain penicillin and streptomycin at 100 units/ml each, 15% heat-inactivated fetal bovine serum, and 50 μM mercaptoethanol. The lines used in this study are summarized in Table 1.

Cell Fusions. Hybrids expressing two H and three L chains were obtained by fusing Sp1/HL-Ag (Table 1) and Sp2B-BU [the μ chains differ in size (8); the L chains can be discriminated by using isoelectric focusing analysis]. Hybrids secreting three H and three L chains were obtained by fusing P1BU1-Ou with Sp2/HLML′ and Sp2/HL-BU with Sp25/5-1 Ag13 (in both fusions all chains differ in $NaDodSO_4$/polyacrylamide gel electrophoresis). Fusions were performed with 3×10^6 cells of each of the parental lines in the presence of 0.7 ml of 50% (vol/vol) polyethylene glycol 1500 (British Drug House, England) in serum-free Dulbecco's modified Eagle's medium. Cells were divided into 24 1-ml cultures. In most cases cultures with hybrids were cloned before being analyzed for Ig chain loss. This was omitted when fewer than 10 hybrids grew out of 24 initial cultures.

Table 1. Cell lines

Code	Line	Ig secretion	Origin	Ref.
A	X63-Ag8	γ_1, κ	BALB/c myeloma	3
B	P1BU1-Ou	γ_{2a}, κ	BALB/c myeloma	3
C	Sp1/HL-Ag	μ, κ	Hybrid of A	4
D	Sp2/HL-BU	γ_{2b}, κ	Hybrid of A	4
E	Sp2/0-Ag14	None	Hybrid of A	5
F	Sp25/5-1-Ag13	$(\mu, \kappa) + (\gamma_1, \kappa)$	Hybrid of A	6
G	Sp6/HLGK	$(\mu, \kappa) + (\gamma_1, \kappa)$	Hybrid of A	4
H	Sp2/01-Ag	None	Hybrid of E	
K	Sp2/HLML′-Ag	$(\mu, L) + (\gamma_{2b}, \kappa)$	Hybrid of D	7
L	Sp2B-BU	$(\mu, L) + (\kappa)$	Hybrid of D	8

Ag, BU, and Ou stand for resistance to 8-azaguanine at 20 μg/ml, 5-bromo-2′-deoxyuridine at 30 μg/ml, and 5 mM oubain, respectively.

Analysis of Chain Loss. Soft agar cloning, radioactivity incorporation, $NaDodSO_4$/polyacrylamide gel electrophoresis, and isoelectric focusing using reduced radiolabeled culture supernatants were performed as described (4, 8).

RESULTS

The chain loss of many different hybrids is summarized in this section. Because many of the original hybrids were obtained by using the X63-Ag8 line, care was taken to include hybrids made by other lines (Sp2/HL-BU; P1BU1-Ou) as well. No difference was observed in their chain loss pattern.

Expression of Immunoglobulin H and L Chains. Loss of Ig-chain expression of randomly picked clones was measured by analyzing labeled and reduced culture supernatants on $NaDodSO_4$/polyacrylamide gel electrophoresis. Fig. 1 shows the pattern obtained from the (D–F) hybrid (see Table 1), which

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Abbreviations: H chain, heavy chain; L chain, light chain.

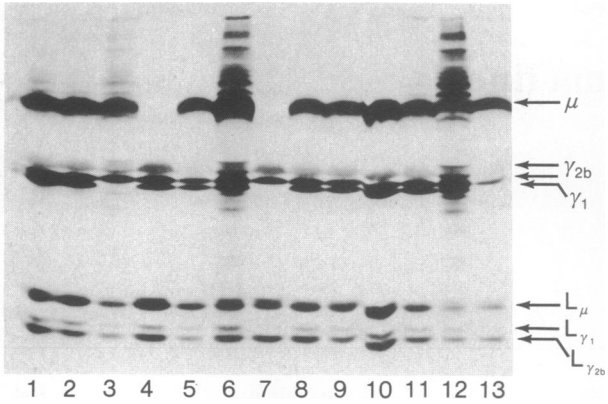

FIG. 1. Hybrid D–F expressing three different Igs. [^{14}C]Leucine-labeled culture supernatants of 13 clones were analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis under reducing conditions. The H chain suffixes given to the L chains indicate their original association.

secretes three different Igs. It can be observed that 2 out of 13 clones lost the secretion of the μ chain and that 3 clones lost the secretion of the γ_1 chain.

These losses are included in group I of Table 2. In this group, for example, two different hybrids (lines) expressing three immunoglobulins were characterized. Ten clones and 182 isolates of these hybrids were analyzed as 33 H chain and 9 L chain losses (observed values). From those, 12 H and 7 L losses were independently obtained. This means that multiple losses of the same H and L chain detected in the isolates of one clone will score as only one independent loss of this particular chain. This avoids the problem of repeats, which may obscure the results (see the L chain losses in group VI: six of the eight observed losses came from one clone). The expected value is based on the independent numbers, which in the example of group I is 9.5 each for H and L, assuming random chain loss.

Chain loss was random among L chains (mostly κ class) and among H chains, irrespective of H chain class, but not when L or H chain losses were compared to each other. Analysis of many different hybrid lines (Table 2) shows that H and L chains are lost randomly only in combinations in which there is a greater number of active genes for L chains than for H chains, except for the highest combination (3H + 3L). All other combinations that expressed a number of L chains equal to or less than the number of H chains showed preferential H chain loss.

Table 2. Summary of Ig chain losses

Group	Lines/ clones/ isolates	Chain combination	Observed/independent and (expected) loss of H	Observed/independent and (expected) loss of L
I	2/10/182	3H 3L	33/12 (9.5)	9/7 (9.5)
II	2/4/70	2H 3L	5/3 (3.6)	17/6 (5.4)
III	1/1/11	1H 3L	1/1	1/1
IV	1/2/26	0H 3L	—	2/2
V	2/5/98	[3H 2L]	15/8 (4.8)	0/0 (3.2)
VI	12/17/647	[2H 2L]	46/24 (13.5)	8/3 (13.5)
VII	6/6/446	1H 2L	5/5 (4.3)	11/8 (8.7)
VIII	8/9/116	[1H 1L]	9/7 (3.5)	0/0 (3.5)

Group I, hybrids between D–F and B–K (for code of letters see Table 1); group II, D–F and C–L; group III, D–F; group IV, C–L; group V, as I; group VI includes groups I, II, and A and D times mouse lymphocytes; group VII, A times mouse lymphocytes; group VIII, E and H times mouse lymphocytes. The expected number is based on the independent losses (see text) and gives the values of random loss of H and L chain expression. The boxed chain combinations gave values not compatible with random loss of H and L chain expression (χ^2 test, one degree of freedom, probability level 5%).

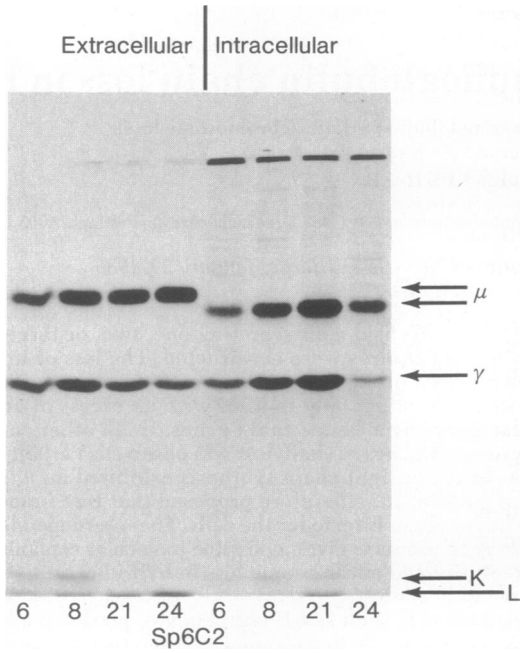

FIG. 2. Differential secretion of K chains in clones of the hybridoma line Sp6C2 (left). Detectable amounts of K were found intracellularly (right) after NaDodSO$_4$/polyacrylamide gel electrophoresis under reducing conditions.

Alteration in H or L Chain Synthesis Changes Chain Loss Pattern. The line Sp6/HLGK secretes, in addition to the myeloma X63-Ag8 γ (G) and κ (K) chains, μ (H) and κ (L) chains with anti-trinitrophenyl specificity (4, 8). Two types of reclones exist for this line: those that secrete normal amounts of K (Fig. 2, Sp6C2/6 and 8) and those that secrete very little K (Fig. 2, Sp6C2/21 and 24). Both types show easily detectable intracellular K chains (9). Random reclones of all four lines show preferential H chain loss: 14 and 32 out of 125 reclones analyzed showed loss of the μ and γ_1 chain, respectively. No light chain loss was observed. In this screening a variant of μ chain was isolated twice out of the 42 reclones analyzed from the Sp6C2/6 line. The variant μ of Sp6C2/6-43 was about 10,000 daltons smaller than wild type μ. Its IgM was multimeric and had anti-trinitrophenyl activity but did not have the light chains covalently bound. When 15 subclones of this variant were analyzed for chain loss, 2 had lost K chain production. This behavior was quite unlike that of the parental lines and group VI in Table 2.

Selection for specificity loss in two independent Sp6/HLk clones (lower-case k symbolizing the phenotype of clones secreting only small amounts, similar to Sp6C2/21 and 24 in Fig. 2), showed that H chain loss was about 1000 times more frequent than L chain loss (9). However, when a similar selection was performed on G-loss variants of Sp6C2/6 and 8 (Fig. 2), H and L were lost equally often, following the pattern of group VII in Table 2.

DISCUSSION

Hengartner *et al.* (1) showed directly that mouse H or L chain loss was correlated with the loss of one copy of chromosome 12 or chromosome 6, respectively. Here we have demonstrated that H and L chain losses, which probably result from losses of their corresponding coding chromosomes, are random unless an excess number of H chains over L chains is expressed by the

cell, a condition that seems to be deleterious to the cell. This restriction seems, however, to relax at higher chain combinations (3H and 3L, Table 2). Perhaps the two remaining L chains make enough product to be equivalent to that of the three H chain genes. This would require a 1.5-fold molar excess of L over H chains, a value well in agreement with measurements in myeloma and mouse lymph node cells (10). The restriction becomes prominent at 2H and 2L and is quite severe in 1H + 1L combination, which is the normal myeloma situation, as Coffino and Scharff (2) have analyzed in detail for the MPC-11 line and Cowan *et al.* (11) for the P3 line. In screening several hundred thousand cells they never found a producer of free H chain [except when the H chain was itself modified (11, 12)], although L chain losses occurred after H chain loss at a frequency of 4×10^{-3} per cell per generation (2). Similar observations have now been made with several hybridoma lines expressing only 1H and 1L (group VIII of table 2; ref. 4).

Could the apparent nonrandom chain and chromosome loss be explained by a deleterious gene dosage effect of a product of chromosome 12 other than the free H chain itself? This is unlikely, as indicated by a series of observations. First, the pattern of chain and chromosome losses is best explained by an interdependence of chromosome 12 and 6. The chromosomes not expressing H and L chains, known to be present in uncertain numbers, but at least once (1), seem not to randomize the results. Second, deletions in the region of the first constant H chain domain alter the chain loss pattern (Sp6C2/6-43 cells). From a similar deletion variant of the MPC-11 (IgG_{2b}) line, Morrison (12) isolated a cell line that produces only H chains, and Milstein and coworkers (11, 13) isolated such a line from another deletion variant line, derived from P3 (IgG_1). At least some of these deletions seem to mimic those found in human H chain disease, in which variant H chains are made in the absence of L chains (14). Third, changing the amount of L chain being secreted again seems to change the normal random H + L loss pattern of the group VII type (Table 2; ref. 9). Exceptions to the idea of free H chains being toxic to the cell have been reported (15). It is interesting that pre-B cells seem to make free μ chains without secreting them (16). I would suggest that in these cases variant H chains are produced.

The hypothesis of chromosomes being interrelated by some of their gene products leads to an apparent directed chromosome loss in the hybrids studied. It may well be that other such interdependencies operate, implying that "pathways" of chromosome losses may exist in hybrid cells. This could explain why in one fusion some hybrids are difficult to grow and others are not. The poor growers may have started with an early random chromosome loss that leads, possibly only after several other chromosome losses, to an incompatible chromosomal complement.

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