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Immunology

Immunoglobulin heavy chain toxicity in plasma cells is neutralized by fusion to pre-B cells

(immunoglobulin chain loss/isoelectric focusing of heavy chain)

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ABSTRACT A plasma cell hybridoma frequently loses its immunoglobulin heavy (H) chain spontaneously but rarely is production of its light (L) chain lost. Upon fusion to a pre-B-cell hybridoma that produces no Ig chain, the L chain is frequently lost. In cells without the L chain the H chain, which is derived from the plasma cell, is not chemically modified. Our results indicate that, in pre-B cells, but not in plasma cells, there must be a mechanism that neutralizes the toxic effect of free H chain.

Myelomas and hybridomas derived from plasma cells secrete immunoglobulin consisting of heavy (H) and light (L) chains. From such cell lines, subclones that have lost H chain expression can be recovered, but they still secrete the L chain (1, 2). The L chain can then be lost at the same frequency as the H chain. On the other hand, it seems to be very difficult to recover cells that have lost L chain expression but that still synthesize the H chain (1–3) except when the cells are mutagenized (4) or express a mutant H chain (5–7). These observations have led to the view that the free H chain is toxic to the cells (2, 3). However, cells of an earlier differentiation stage, pre-B cells, synthesize intracellular H chain in the absence of the L chain (8). To explain this difference, several possibilities have been proposed: (*i*) the rate of H chain synthesis in pre-B cells is too low to damage the cell (9); (*ii*) the pre-B cell synthesizes a different H chain that is not toxic to the cell (2); or (*iii*) in pre-B cells, there is a special protein that neutralizes the toxic effect of the free H chain (10, 11).

Here we report that hybridomas derived from pre-B cells are not different from plasma cell hybridomas with respect to their rate of H chain synthesis and steady-state level of H chain. However, they do not synthesize the L chain. In consequence, we wanted to answer the question whether there are pre-B-cell hybridomas that can synthesize a free H chain, which has been shown to be toxic in plasma cell hybridomas.

MATERIALS AND METHODS

Cell Lines. Sp2 and GK14.1 were established and provided by G. Köhler (Basel). The cell lines are derived from fusions between spleen cells and a myeloma, X63 Ag8, and synthesize IgG2b. Sp2.0 is an azaguanine-resistant subclone of Sp2 and has lost Ig expression. Cell lines H32-21, H32-3, H32-8, and H6 are derived from fusions between γ2b-synthesizing subclones of the Abelson virus-transformed pre-B-cell line 18-81 and X63 Ag8653 (12). Clone H62 is a subclone of H6 and synthesizes no Ig chain. NORA hybridomas were made by fusion of Sp2 HL Ag14 and H62. SPSP hybridomas are derived from a fusion between Sp2.0 and Sp2. Cell fusion was carried out as described (12). The genealogy of the various hybridomas is given in Fig. 1.

Isolation of Subclones with Ig Chain Loss. Soft agar cloning and antiserum overlay was carried out according to the method of Coffino and Scharff (1). When an antiserum against IgG2b (γ2b,κ) was used, about 1% of the cells formed colonies without precipitation. These clones were isolated, and their Ig expression was analyzed by immunofluorescence. Clones of interest were grown in mass culture and further analyzed by immunoprecipitation and electrophoresis.

NaDodSO$_4$/Polyacrylamide Electrophoresis. Cell labeling, immunoprecipitation, and NaDodSO$_4$/polyacrylamide gel electrophoresis were carried out as described (12). Pulse labeling (30 min) was carried out by the addition of [^{35}S]methionine to cells that had been incubated in methionine-free select medium/10% dialyzed fetal calf serum for 1 hr. The amount of [^{35}S]methionine incorporated into the precipitated proteins was measured after gel fractionation by scintillation assay of the solubilized gel slices.

Isoelectric Focusing. [^{35}S]Methionine-labeled Ig precipitates were dissolved in 9.5 M urea/2% Nonidet P-40/2% Ampholine (pH 5–11)/5% 2-mercaptoethanol and applied to isoelectrofocusing slab gels. The gel composition was according to O'Farrell (13). Electrophoresis was carried out for 1 hr at 250 V, for 12 hr at 400 V, and finally for 1 hr at 800 V. The proteins were visualized by fluorography.

Immunofluorescence. The purification and fluorochrome conjugation of goat antibodies specific for mouse H chain isotypes and the methods for immunofluorescence detection of intracellular Ig have been described (14).

RESULTS

The Rate of Ig H Chain Synthesis and the Steady-State Level of the H Chain in Plasma Cell- and in Pre-B Cell-Derived Hybridomas Are of the Same Order of Magnitude. The pre-B cell is characterized by the synthesis of intracellular H chain in the absence of the L chain. The free H chain is toxic in plasma cells, but pre-B cells may survive because they synthesize the H chain in small amounts (15, 16) that would not damage the cell. To test this hypothesis, we increased the amount of free H chain present in pre-B cells by fusion to a myeloma. The steady-state level of RNA specific for the H chain is the same in pre-B-cell hybridomas and in plasma cell hybridomas (16).

By measuring the short-term incorporation of radiolabeled methionine into the H chain (Fig. 2), we have compared the rate of Ig H chain synthesis in pre-B-cell-derived hybridomas with that of plasma cell-derived hybridomas. The amount of radioactivity incorporated into the various H chains was of the same order of magnitude. Also, the steady-state level of the H chain, as determined by long-term label-

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Abbreviations: H chain and L chain, heavy and light chain, respectively, of Ig; BiP, H chain binding protein.

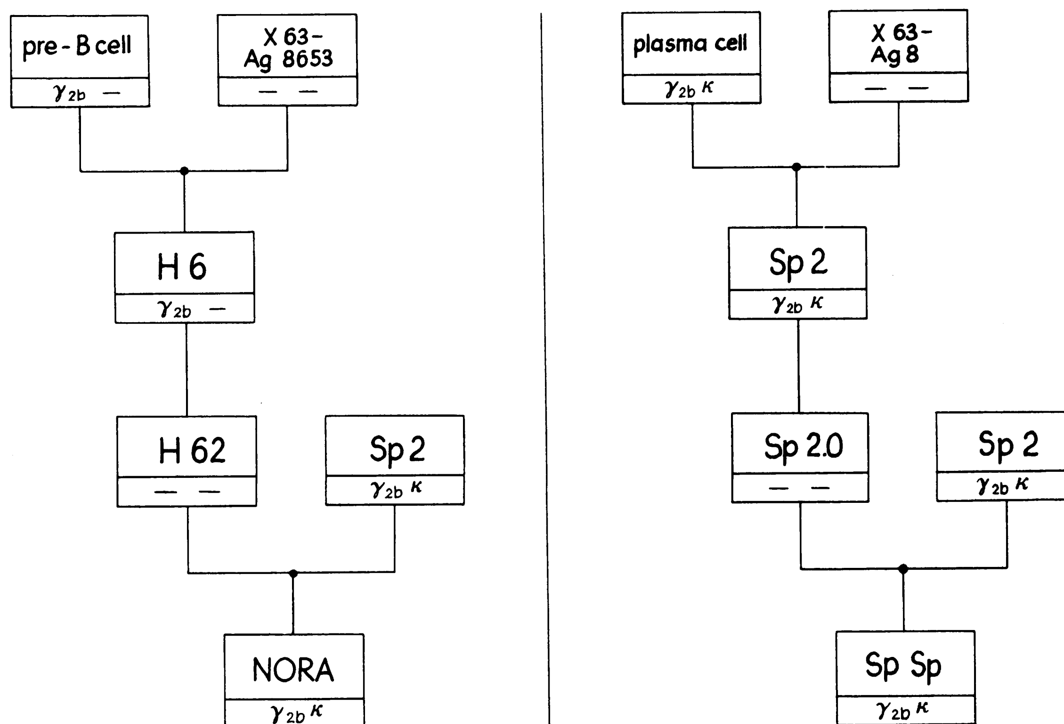

FIG. 1. Genealogy of selected hybridomas derived from a pre-B cell, or from a plasma cell, or from both. The kind of Ig chain synthesized is given below the clone designation.

ing (Fig. 3) and by immunofluorescence intensity (data not shown), was not different. This does not formally exclude that pre-B cells survive the expression of free H chain because of a low rate of synthesis, but there would still remain the question of why the H chain is not toxic for pre-B-cell-derived hybridomas as it is for plasma cell-derived hybridomas.

A Given H Chain Is Toxic in Plasma Cells but Not in Pre-B Cells. Because pre-B-cell-derived hybridomas can survive high levels of the H chain, we wanted to know whether they are able to survive the expression of a H chain, the toxicity of which has been demonstrated in plasma cells. For that purpose, we fused a plasma cell hybridoma to a pre-B-cell hybridoma that had lost its own H chain expression.

The Sp2 cell line is derived from fusion of a plasma cell with a myeloma (Fig. 1). It synthesizes both H and L chain and exhibits the H chain toxicity phenomenon (2). We confirmed this by recovering Ig chain-loss variants according to the method of Coffino and Scharff (1). Of 74 subclones, 69 expressed no H chain, one expressed no L chain (that is, expressed H chain only), and 4 did not express any Ig chain at all (Table 1). We then fused the Sp2 cell line to a pre-B-cell-derived hybridoma, H62, that had lost the expression of its own Ig chain (Fig. 1). The resulting hybrid cell line NORA 4 secreted H and L chain from the Sp2 parent line (Figs. 3 and 4). The NORA cell line tolerates the H chain in the ab-

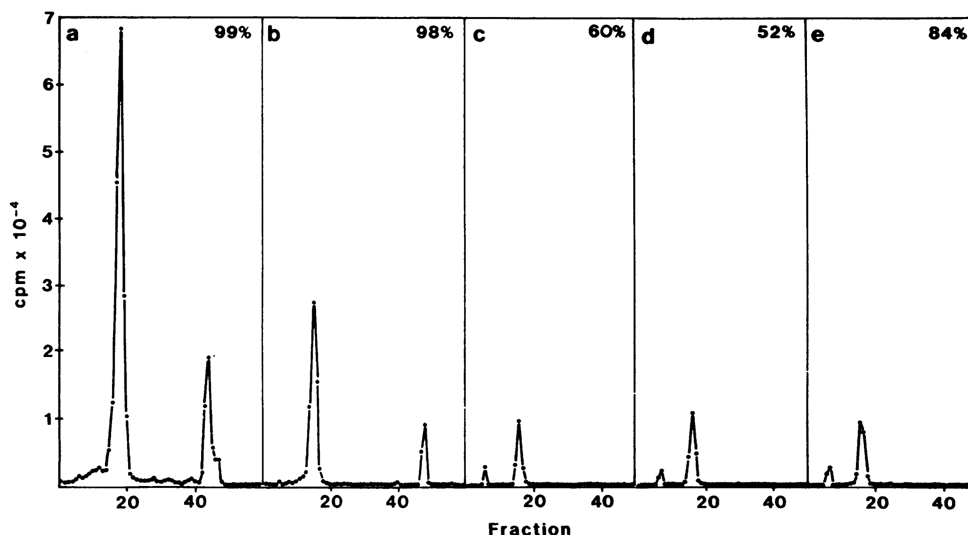

FIG. 2. Amount of radioactivity incorporated into immunoprecipitated proteins after separation on polyacrylamide gel. GK14.1 (*a*) and Sp2 (*b*) are plasma cell-derived hybridomas synthesizing both H and L chain; H32-21 (*c*), H32-3 (*d*), and H32-8 (*e*) are pre-B-cell-derived hybridomas synthesizing a H chain that is associated with BiP (11). The indicated percentages of cells synthesizing Ig were determined by immunofluorescence.

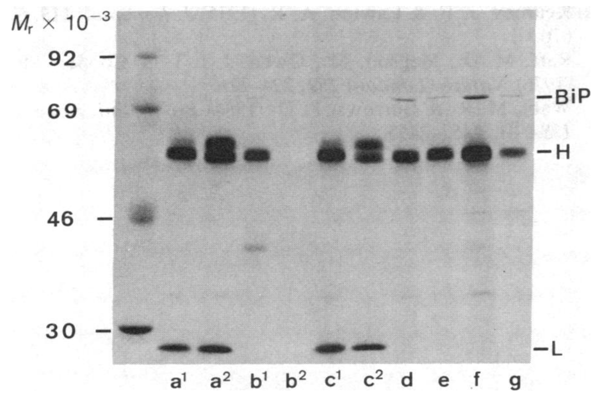

FIG. 3. NaDodSO4/polyacrylamide gel electrophoresis analysis of the Igs of various hybridomas synthesizing both H and L chain or synthesizing the H chain only. Sp2 (lanes a) and NORA 4 (lanes c) secrete both H and L chain; SPSP 1.55 (lanes b), NORA 4.2 (lane d), NORA 4.16 (lane e), NORA 4.8.20 (lane f), and the pre-B-cell hybridoma H 61 (lane g) synthesize the H chain without the L chain but do not secrete it.

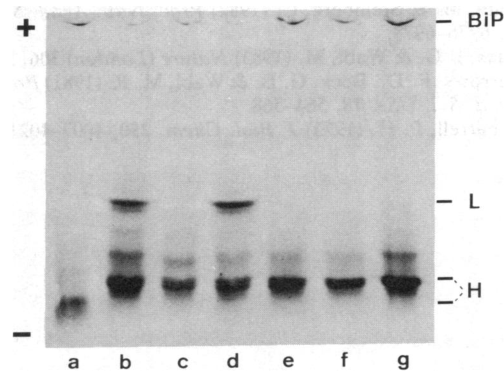

FIG. 4. Isoelectric focusing analysis of intracellular Ig of various hybridomas synthesizing both H and L chain or synthesizing H chain only. Lanes: a, pre-B-cell hybridoma H 61 (γ2b); b, Sp2 (γ2b,κ); c, SPSP 1.55 (γ2b); d, NORA 4 (γ2b,κ); e, NORA 4.2 (γ2b); f, NORA 4.16 (γ2b); g, NORA 4.8.20 (γ2b). The γ2b chain of H 61 has a variable region different from the one of the Sp2 cells and their hybridomas.

sence of the L chain. Ten out of 55 subclones of NORA 4 with Ig chain loss synthesized Ig H chain without the L chain, 42 synthesized the L chain only, and 3 did not synthesize any Ig chain at all (Table 1). A subclone of NORA 4, NORA 4.8, that synthesizes both H and L chain, showed the same chain loss distribution (Table 1). In no instance was the H chain secreted. As a control to account for the increased chromosome numbers of the NORA 4 hybrid as compared with Sp2, we fused the Sp2 cell line to a nonproducing subclone, Sp2.0, generating SPSP hybridomas (Fig. 1). From these hybridomas, subclones synthesizing free H chain should be rare. Indeed, of 125 subclones with Ig chain loss, we recovered only 1 subclone expressing free H chain (Table 1). Of these subclones, 122 had lost the H chain and two had lost both Ig chains. Comparison of the H chain of hybridomas synthesizing both H and L chain or the H chain alone revealed no difference in size (Fig. 3) nor in isoelectric focusing behavior (Fig. 4). Since in this case, the H chain is one that is known to be toxic, we conclude that in pre-B cells there is a mechanism neutralizing the toxic effect of free H chains.

DISCUSSION

Why are free Ig H chains toxic in plasma cells but not in pre-B cells? From the plasmacytoma MOPC 21, a variant could be recovered that synthesizes only the H chain. The mutant H chain of this variant lacks the last 67 carboxyl-terminal amino acids and forms polymers of at least 20 H chains (9). This polymerization is probably due to free sulfhydryl (SH) groups on the H chain that would normally form the L-H bridge. The normal H chain probably could also polymerize in the absence of L chains but would form much larger insoluble complexes that would damage the cell. Polymerization of L chains cannot occur because no additional free SH groups are available once the L chain has formed a dimer. It is of interest that H chains are predominantly found as monomers or dimers in pre-B cells (11), as well as in the H chain-synthesizing subclones of the NORA 4 hybrid line (data not shown). Thus, neutralization of the toxic effect of the free H chain may be achieved by the prevention of H chain polymerization—for example, by an enzyme that alters the reactive SH groups of the Ig H chain. Since we did not find any difference in the isoelectric focusing pattern of the intracellular H chains of Sp2, NORA 4, and of those subclones synthesizing no L chain (Fig. 4), one can postulate a protein that either rapidly degrades accumulating free H chain or protects the reactive SH groups without chemical modification. We have already described a protein (heavy chain-binding protein, BiP) that binds to Ig H chains not associated with the L chain (11). In all NORA subclones that have lost the L chain (some of them are shown in Fig. 3), the H chain is associated with the BiP. If the BiP is neutralizing H chain toxicity it should be less active in plasma cells than in pre-B cells.

Whatever the mechanism for neutralization of H chain toxicity, our results demand an explanation of why H chain synthesis precedes L chain synthesis in B-cell ontogeny (10).

Table 1. Ig chain loss pattern in various hybridomas

Hybridoma	No. of subclones H chain lost	L chain lost	H + L chain lost
Sp2	57	0	4
Sp2.68	12	1	0
Total	69	1	4
NORA 4	42	10	3
NORA 4.8	42	12	0
Total	84	22	3
SPSP 1	59	1	2
SPSP 2	63	0	0
Total	122	1	2

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1. Coffino, P. & Scharff, M. D. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 219–223.
2. Köhler, G. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2197–2199.
3. Wilde, C. D. & Milstein, C. (1980) *Eur. J. Immunol.* **10**, 462–467.
4. Morrison, S. L. & Scharff, M. D. (1975) *J. Immunol.* **114**, 655–659.
5. Morrison, S. L. (1978) *Eur. J. Immunol.* **8**, 194–199.
6. Sonenshein, G. E., Siekevitz, M., Siebert, G. R. & Gefter, M. L. (1978) *J. Exp. Med.* **148**, 301–312.
7. Cowan, N. J., Secher, D. S. & Milstein, C. (1974) *J. Mol. Biol.* **90**, 691–701.
8. Burrows, P. D., LeJeune, M. & Kearney, J. F. (1979) *Nature (London)* **280**, 838–841.
9. Argon, Y., Burrone, O. R. & Milstein, C. (1983) *Eur. J. Immunol.* **13**, 301–305.

10. Wabl, M. & Steinberg, C. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6976–6978.
11. Haas, I. G. & Wabl, M. (1983) *Nature (London)* **306,** 387–389.
12. Burrows, P. D., Beck, G. B. & Wabl, M. R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 564–568.
13. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021.
14. Kearney, J. F. & Lawton, A. R. (1975) *J. Immunol.* **115,** 671–676.
15. Raff, M. D., Megson, M., Owen, J. J. T. & Cooper, M. D. (1976) *Nature (London)* **259,** 224–226.
16. Wabl, M. R. & Burrows, P. D. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2452–2455.