Myeloma with multiple rearranged immunoglobulin kappa genes: only one kappa gene codes for kappa chains

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Received 21 July 1980

ABSTRACT

In many myelomas more than one kappa gene is rearranged (2-5). We are reporting here the results of studies undertaken to determine whether all the rearranged genes are expressed. It was found that in the myeloma NS-1 three different rearranged kappa genes exist. In a subline of NS-1 and several hybridomas produced by fusion of mouse spleen cells with NS-1 it was found that production of NS-1 kappa chains was correlated with the presence of one of the three kappa genes. Loss of this "expressed" gene eliminated the synthesis of the NS-1 kappa chains, loss of one of the other two rearranged kappa genes did not. It is hypothesized, that allelic exclusion (20) of kappa genes generally operates by the functional rearrangement of one kappa gene; other rearrangements are relatively frequent, at least in myelomas, but mostly they are nonfunctional and thus scrambled antibody molecules do not arise.

INTRODUCTION

It has generally been observed that myeloma cells produce only one type of immunoglobulin (1). However recently it has become apparent that rearrangements of immunoglobulin genes in myeloma cells are not limited to one gene in a given class (2-5). As a matter of fact, the majority of myelomas may lack kappa genes in germline configuration (3). It is therefore of interest to determine whether only one or all the rearranged kappa genes of a cell are expressed on the protein level. We have now investigated this question in the myeloma NS-1, a subline of MOPC-21 (6). NS-1 possesses three rearranged C_{κ} genes (7), i.e. it was found that when the DNA of this myeloma is digested with the restriction enzyme BamH-1 and the resulting DNA fragments are analyzed by Southern blotting (8) and hybridization with a cloned C_{κ} probe, three C fragments of 6.9kb, 5.9kb, and 5.2kb are seen (7). (Note that the fragment sizes have been slightly revised due to greater accuracy of radiolabeled standards included in the blots (18) - they correspond to the previously listed fragments of 7.3kb, 6.5kb, and 5.5kb.) Since BamH-1 does not cut within the C_{κ} region (3) three different C_{κ} genes must

be present in NS-1. All three are rearranged, since germline C_{κ} genes of the Balb/c mouse give a single C_{κ} band of 12.9kb (3). It is not known whether the three genes are due to triploidy of chromosome six (10) in NS-1, or whether the three rearranged genes are the manifestation of at least two C_{κ} genes per haploid set of normal mouse cells (11). Lastly, it may be possible that in this myeloma a gene duplication has taken place on one of the copies of chromosome six. [But see discussion of companion article (18).]

The observation of a variant of NS-1 which had lost the production of kappa chains made it possible to investigate the function of the three C_{κ} genes. In addition we investigated several hybridomas derived from NS-1. The DNAs of these various cell lines were analyzed by restriction enzyme digestion and the kappa chains produced by the cells were determined by radioimmunoprecipitation of metabolically labeled proteins. Thus the presence of certain rearranged C_{κ} genes could be correlated with the ability of the cells to synthesize the NS-1 type kappa chains.

MATERIALS AND METHODS

Culture of myeloma and hybridoma cells. The cells were grown in tissue culture as described by Yeh et al. (12).

Preparation of mouse DNA, restriction enzyme digestion and Southern blotting. These procedures were carried out as described (3,8).

Determination of immunoglobulin synthesis by myeloma and hybridoma cells. The tissue culture lines were labeled with ^{35}S methionine as described (13). Briefly, the cells were labeled with ^{35}S methionine for three hours, and the immunoglobulins were precipitated from culture supernatants (S) and cell lysates (C) by a rabbit anti mouse kappa antiserum (13). SDS-PAGE analysis was done on the redissolved immunoprecipitates as described (13).

RESULTS

Figure 1A (NS-1,C) shows that NS-1 produces kappa chains in the cytoplasm (but no associated H chains). As has been described (14) NS-1 does not secrete the kappa chains (Fig. 1A, NS-1,S). On the other hand, a variant, NS-1n, which arose spontaneously in our laboratory, does not synthesize any kappa chains any more (Fig. 1B). Two different samplings of NS-1n were analyzed, the same cell numbers were used as in Fig. 1A for NS-1; the NS-1n cells produced the same levels of total proteins as the other cells (data not shown).

Figure 1. Immunoglobulins synthesized by NS-1 myeloma as well as a variant and hybridomas derived from NS-1. A and B are radioautographs of two separate gels. NS-1: nonsecreting myeloma (14). NS-1n: two different samplings from an NS-1 variant which arose in our laboratory. H-11, F-5, and C-3: three different hybridomas derived by fusion of NS-1 with Balb/c spleen cells. F-5 and H-11 produce IgG-antibodies to human lung carcinomas (Yeh and Hellström, unpublished). The C-3 hybridoma (previously named 3.1) secretes antibodies specific for a human melanoma (12). G = IgG. k = kappa.

The DNAs of NS-1 and NS-1n were digested with the restriction enzyme BamH-1 and analyzed in Southern blots (8). The hybridization probe used was the C region and 3' untranslated region of kappa genes isolated from a plasmid clone containing kappa cDNA (15) prepared from the kappa mRNA of the myeloma MOPC-167. (The clone was obtained from P. Early, Cal. Tech. The preparation and nick translation of the C_k probe from the clone has been described (3).) When the DNA of NS-1n was compared with the DNA of NS-1 it was found that it had lost the 5.2kb BamH-1 C_k gene (the "5.2kb gene"), but retained the 6.9 and 5.9kb genes (Fig. 2). It appeared that the 6.9 and 5.9kb genes do not code for kappa chain synthesis. This was confirmed in other tumors: the hybridoma Sp-2 is a nonproducing variant derived from the fusion of a spleen cell with the myeloma X63-Ag8, another subline of the myeloma MOPC-21, from which NS-1 was derived independently (16,17). The restriction enzyme-Southern blot of Sp-2 is identical to NS-1n, i.e. it has also lost the 5.2kb C_k gene and retained the 5.9 and 6.9kb genes (Table 1).

Figure 2. Autoradiogram of Southern blots of myeloma and hybridoma DNAs. Restriction enzyme digestion with BamH-1, Southern blot analysis (8) with a ^{32}P nick translated (9) cloned C_κ probe (3). Fragment sizes were determined relative to an internal standard^k of HindIII cleaved phage lambda DNA. 1 = MOPC-21 myeloma, 2 = NS-1 myeloma, 3 = NS-1n myeloma, 4 = Sp2 hybridoma, 5 = C-3 hybridoma, 6 = F-5 hybridoma, 7 = H-11 hybridoma. a = 6.9kb, b = 5.9kb, c = 5.2kb.

The Figure represents several different Southern blots; therefore the fragments do not always perfectly align.

Thus these completely independently derived variants have both lost the ability to synthesize kappa chains concurrently with the loss of the 5.2kb gene.

The dependence of the synthesis of the NS-1 kappa chain on the presence of the 5.2kb gene is further supported by the findings in three hybridomas produced by Yeh et al. (12). The hybridomas H-11 and F-5 both produce the NS-1 kappa chains, as well as kappa chains of higher mobility coded for by the spleen cell partner (Fig. 1A). Both these hybridomas possess the 5.2kb gene, but F-5 lacks the 6.9kb gene (Fig. 2). Thus again it appears that the 6.9kb gene is not required for the NS-1 kappa chain synthesis. On the other hand, the hybridoma C-3 does not produce the NS-1 kappa chain, it only shows a single kappa chain type of a slightly greater mobility than the NS-1 kappa chains (Fig. 1B). This hybridoma lacks the 5.2kb gene (Fig. 2), again supporting the idea that this is the only NS-1 C_κ gene which codes for the NS-1 kappa chains.

DISCUSSION

Table 1 summarizes the data and shows a clear correlation between the

Table 1. NS-1 type C_k genes and kappa chain synthesis in the myeloma NS-1 as well as a variant and hybridomas derived from it.

Tumor	NS-1 type C_k fragments	Synthesis of NS-1 type kappa chains
	in BamH-1 digested DNA ^a	
	kb	
NS-1 myeloma	6.9	
	5.9	
	5.2	+
NS-1n myeloma	6.9	
	5.9	-
Sp-2 hybridoma	6.9	
	5.9	- ^b
C-3 ^c hybridoma	6.9	
	5.9	-
F-5 ^c hybridoma	5.9	
	5.2	+
H-11 ^c hybridoma	6.9	
	5.9	
	5.2	+

Liver	germline C_k	
	12.9	

^a Data from Figure 2. The size of the restriction fragment of liver DNA is taken from Wilson et al. (3).

^b The absence of kappa chain synthesis by the hybridoma Sp-2 has been shown by Shulman et al. (17).

^c The hybridomas have additional C_k genes derived from the spleen cell partner (7) which are not listed here.

presence of the 5.2kb C_k gene and the synthesis of NS-1 kappa chains. The 6.9kb and 5.9kb genes are therefore nonfunctional with respect to the synthesis of NS-1 kappa chains. The basis for the nonfunction of certain rearranged kappa genes is now being determined in our laboratory (see also the accompanying article, ref. 18). We have shown by DNase I susceptibility that nonfunctional rearranged C_k genes are present in a similar chromatin conformation to functionally rearranged C_k genes (19), i.e. the chromatin state is altered compared with kappa genes in germline context. Thus the nonfunctional rearranged genes are inhibited at a later step of gene ex-

pression: it may be transcription, posttranscriptional processing of precursor RNA, or translation.

It is not known whether normal B cell precursors experience nonfunctional kappa gene rearrangements, and if so, what the relative proportions of functional and nonfunctional kappa gene rearrangements are. Each normal plasma cell of an allotype heterozygous individual produces only immunoglobulin chains coded for by one of the alleles: "allelic exclusion" (20). It is possible, that this phenomenon of allelic exclusion depends simply on the selection of a single functional rearrangement. The observation that most B cells possess one germline C_k gene (3,7,21 and Selsing and Storb, unpublished), suggests that the two alleles of kappa genes do not both undergo rearrangement in the same cell at the same time, i.e. rearrangement occurs generally only on one chromosome. However, additional rearrangements may occur. Since two different functional rearrangements would lead to scrambled antibody molecules only the combination of a functional rearrangement with a germline gene, or a functional with a nonfunctional rearrangement would be successfully expressed after selection by antigen.

ACKNOWLEDGEMENTS

We thank M. Yeh and J. Brown in I. and K.E. Hellström's laboratory for the hybridomas C-3, F-5, and H-11, C. Milstein for NS-1, M. Shulman, C. Wilde, and G. Köhler for the hybridoma Sp-2, P. Early in L. Hood's laboratory for clone pk167R1 and K. Spangler and R. Finical for typing of the manuscript. This work was supported by research grants from NIH (CA/AI 25754 and DE 02600) and NSF (PCM 78-13205).

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