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Immunology

Preferential expression of variable region heavy chain gene segments by predominant 2,4-dinitrophenyl-specific BALB/c neonatal antibody clonotypes

(immune repertoire/variable region gene families/DNA sequence)

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ABSTRACT **The B-cell repertoire in neonatal mice contains predominant clonotypes that are reproducibly expressed at particular times after birth. We have isolated and sequenced heavy and light chain cDNA clones from three 2,4-dinitrophenyl-specific neonatal hybridomas. Two of these hybridomas (TF2-36 and TF5-139) express idiotypes (Ids) that predominate during the first days after birth, and the third hybridoma (TF2-76) expresses an Id that predominates during the second week after birth. The heavy (H) chain variable (V) region of the TF2-76 hybridoma protein is encoded by a member of the 7183 V_H family, one of eight families of murine V_H genes that have been defined by Brodeur and Riblet [Brodeur, P. H. & Riblet, R. (1984) *Eur. J. Immunol.* 14, 922–930]. Members of this family have been found to undergo a disproportionately high frequency of rearrangement in fetal and neonatal liver pre-B-cells. Because the 7183 V_H family is located close to the H chain joining (J) region gene segments, J_H, other workers have proposed that V_H rearrangement frequency is related to distance from the J_H segments. However, the two earlier-appearing predominant clonotypes expressed by TF2-36 and TF5-139 hybridoma proteins utilize a member of the 36-60 V_H family, probably V_H 1210.7, which is located distal to the J_H gene segments on chromosome 12. Since 20–30% of day 3 dinitrophenyl-specific B cells express either the Id(TF2-36) or the Id(TF5-139), the V_H 1210.7 gene must be utilized at high frequency early in development. These results indicate that the utilization of rearranged V_H segments is strongly influenced by factors other than distance from J_H.**

Although the adult primary B-cell repertoire of BALB/c mice exceeds 10^7 clonotypes (1, 2), the antibody repertoire at birth is limited to 10^4–10^5 different specificities (3, 4). During the first weeks after birth, this repertoire undergoes a reproducible and patterned expansion (3–9). Thus, in the first 3 days of neonatal life, all BALB/c neonates share three predominant 2,4-dinitrophenyl (DNP)-specific and three predominant 2,4,6-trinitrophenyl (TNP)-specific clonotypes (3). The same mice do not express predominant clonotypes for fluorescein (3), phosphocholine (6), and either $\alpha(1\rightarrow3)$ or $\alpha(1\rightarrow6)$ dextran determinants (7, 8). Phosphocholine-specific clones reproducibly appear several days after DNP- or TNP-specific clones in both fetal liver (9) and neonatal spleen (6). Two weeks after birth, the antibody repertoire, which contains $\approx10^6$ specificities, is still more restricted than the adult repertoire (5).

Recently, several investigators have attempted to correlate this patterned acquisition of the antibody repertoire with the preferential rearrangement of specific variable (V) region gene segments (10, 11). Murine germ-line heavy (H) chain V region gene segments (V_H) have been grouped by sequence homologies into eight families (12, 13). V_H recombinant strains have been used to obtain the order of these V_H families, whose names relate to cell lines, on chromosome 12 as: 5′ (V_H 3609, V_H 36-60, V_H J606, V_H X24)–V_H J558–V_H S107–V_H Q52–V_H 7183–*D*–J_H 3′ (14). The relative order of the set of V_H gene families 5′ to the J558 V_H family cannot be mapped with the existing recombinant strains. The potential correlation between sequential rearrangement of *V* segments and sequential expression of antibody specificities is based on the observation that the rearrangement of V_H families in fetal liver pre-B-cells is not random (10, 11). Cells with a rearranged member of the 7183 V_H family have been the most frequently found among Abelson murine leukemia virus (Ab-MuLV)-transformed pre-B-cells and among fetal liver pre-B-cell hybridomas. This is especially striking because the 7183 V_H family represents only $\approx10\%$ of all V_H genes (13). One member of the 7183 V_H family, V_H 81X, rearranges at a particularly high frequency. V_H 81X, one of the most J_H-proximal V_H regions, is rearranged in almost half of Ab-MuLV-transformed fetal liver pre-B-cells (10).

In order to evaluate the contribution of such a developmentally ordered rearrangement of *V* segments to the repertoire of antigen-responsive B cells, we have examined the *V* genes utilized by three hybridomas derived by fusion of DNP-hemocyanin-stimulated neonatal spleen cells (4). The idiotypes expressed by the antibody products of each of these three hybridomas have been shown to be expressed independently on a high proportion of the anti-DNP monoclonal antibody products of BALB/c neonatal spleen cells (4). Our findings provide a link between *V* rearrangement and expression in the repertoire of antigen-responsive neonatal B cells; they indicate that the position of given V_H segments within the V_H locus may not entirely determine the order of their preferential utilization.

MATERIALS AND METHODS

TF2-76, TF2-36, and TF5-139 hybridomas were made by transfer fusion of neonatal spleen cells with myeloma cells as described in detail (4). The myeloma cell used for the fusion was an early isolate of SP2/0 (15).

cDNAs were cloned from poly(A)$^+$ RNAs (16, 17) by standard methods. For V_H regions, first-strand cDNA synthesis was primed either by the oligonucleotide d(CCCGGG) (at 1.2 μM), which is complementary to the *Sma* I cleavage site located 91 base pairs (bp) from the 5′ end of the constant

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Abbreviations: DNP, 2,4-dinitrophenyl; TNP, 2,4,6-trinitrophenyl; V, variable; C, constant; D, diversity; J, joining; H, heavy; A-MuLV, Abelson murine leukemia virus; Id, idiotype; bp, base pair(s); V_H 36, V_H 139, and V_H 76, V region H chain genes expressed by hybridomas TF2-36, TF5-139, and TF2-76; V_κ 36 and V_κ 139, V_κ genes expressed by TF2-36 and TF5-139.

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(C) region μ chain gene (C_μ) (18), or by the oligonucleotide d(GCCGATTATC) (at 0.6 μM), which is complementary to C_α at nucleotides 66–75 (19). First-strand cDNA synthesis of V_L was primed by oligo(dT).

Second-strand cDNA synthesis was carried out by the loop-back/S1 nuclease procedure (20) or by the method of Gubler and Hoffman (21). Double-stranded cDNAs were hybridized into the *Pst* I site of pBR322 by the homopolymeric tailing procedure (20) and introduced into strain MC1061 (22). Libraries constructed from TF2-76 and TF2-36 mRNAs were screened (23) with a synthetic oligonucleotide d(GCAGGAGACGAG) that hybridizes to nucleotides 31–42 of C_μ or with a restriction fragment from a clone carrying only a portion of the TF2-76 *V* region. Because preliminary work had revealed complete identity between the V_H region genes contained in hybridomas TF2-36 and TF5-139 (V_H 36 and V_H 139) over 82 bp, the TF5-139 library was screened with a restriction fragment from a V_H36 cDNA clone. These libraries yielded cDNA clones pμ76[4], pμ36[21], and pα139[3] from hybridomas TF2-76, TF2-36, and TF5-139, respectively. Clones pκ36[3] and pκ139[17] were isolated from oligo(dT)-primed libraries by screening with a C_κ genomic probe (24).

DNA sequencing was by the Maxam–Gilbert procedure (25) using buffer gradient gels (26).

Hybridoma antibodies TF2-36 and TF5-139 were affinity-purified on DNP-conjugated bovine serum albumin-Sepharose columns and eluted with 0.54 M acetic acid (4). H and L chains were separated by polyacrylamide gel electrophoresis in detergent, and individual bands were stained with Coomassie blue, excised, and electroeluted (27). Automated sequence analysis was performed by Tony Hugli at this Institute, using an Applied Biosystems 470A gas-phase protein sequencer. Phenylthiohydantoin-conjugated amino acids were identified by HPLC (Waters Associates) at 254 nm after separation on Altex Ultrasphere-ODS column (28).

RESULTS

Neonatal Hybridomas Expressing Predominant Anti-DNP Clonotypes. Neonatal hybridomas were previously made from spleen cells of 3-day-old BALB/c mice by transfer fusion (4). Two IgM-producing hybridomas, TF2-36 and TF2-76, were obtained from the same transfer fusion and have been characterized by Denis and Klinman (4). A third hybridoma, TF5-139, which produces an IgA antibody, was obtained from a later fusion. The representation in the neonatal repertoire of the clonotypes expressed by these neonatal anti-DNP hybridoma proteins was assessed by screening DNP-binding fragment culture supernatants with anti-idiotypic antibodies made to each of the neonatal hybridoma proteins (4). As reported previously (4), TF2-36 expresses an idiotype that predominates early, reaching its greatest representation in the B-cell repertoire at 3 days after birth. The frequency of DNP-specific B cells expressing a TF5-139 idiotype was determined only for 3-day-old and adult spleen cells. On day 3, Id(TF2-36) and Id(TF5-139) were expressed by 15% and 10% of DNP-specific B cells, respectively. Id(TF2-76) was expressed at birth and steadily increased to day 12, when it was found on 6% of anti-DNP-specific B cells. Representation of all three idiotypes in the anti-DNP repertoire declined, so that in the adult they were expressed by less than 2% of anti-DNP-specific splenic B cells.

The V_H Region Gene Used by Hybridoma TF2-76 (V_H 76) Is a Member of the 7183 V_H Gene Family. An RNA blot of poly(A)$^+$ RNA from hybridoma TF2-76 and SP2/0 cells was hybridized with a V_H 76 probe and a C_μ cDNA probe (29). Both probes hybridized to a 2.4-kilobase RNA from TF2-76; neither probe hybridized to mRNA from SP2/0 (data not shown). Thus, the pμ76[4] cDNA clone contains the V_H region used by the TF2-76 neonatal anti-DNP hybridoma protein. The nucleotide sequence of the TF2-76 V_H segment from pμ76[4] shows 97% homology with V_H E4.15 and 95% homology with V_H D6.96, both members of the 7183 V_H family (Fig. 1) (10). The signal peptide regions associated with V_H 76 and V_H E4.15 have 86% identity; a comparison of the 5′ untranslated regions associated with V_H 76 and V_H E4.15 showed many insertions and deletions upstream of position −26. We conclude that V_H 76 is a member of the 7183 V_H family, but it is not derived from the V_H E4.15 or V_H D6.96 genes.

V_H 36 and V_H 139 Are Members of the 36-60 V_H Gene Family. cDNA clones containing the V_H region were prepared from TF2-36 (pμ36[21]) and TF5-139 (pα139[3]) poly(A)$^+$ RNA and sequenced. V_H 36 and V_H 139 differ by one nucleotide, resulting in an amino acid change at codon 16 (Fig. 2). When these sequences are compared with other V_H sequences, closest homology is found with V_H 1210.7, a member of the 36-60 V_H family (30). V_H 139 and V_H 36 share a single silent nucleotide difference from V_H 1210.7 at codon 15. V_H 36 also differs from V_H 1210.7 at codon 16. Because RNA blots of poly(A)$^+$ mRNA from SP2/0 do not show bands when hybridized with cloned C_μ and C_α cDNA probes (ref. 29; data not shown), we infer that pμ36[21] and pα139[3] contain the V_H regions used by the TF2-36 and TF5-139 neonatal anti-DNP hybridoma proteins.

***D* and J_H Segments Encoding the Neonatal Hybridoma Antibodies.** While the TF2-36 and TF5-139 antibodies appear to utilize the same V_H segment, they use different *D* and J_H segments (Fig. 3). The TF2-36 antibody uses a *D* segment with closest homology to either $D_{SP2.3}$ or $D_{FL16.1}$ germ-line gene segments (31). If either of these *D* gene segments is used, the *D* segment of TF2-36 contains only the central portion and differs from each by 4 of 10 nucleotides. The *D* segment of TF5-139 has closest homology with the D_{Q52} germ-line gene segment. It also contains two additional base pairs at the *D*–J_H junction. The TF2-76 antibody utilizes a

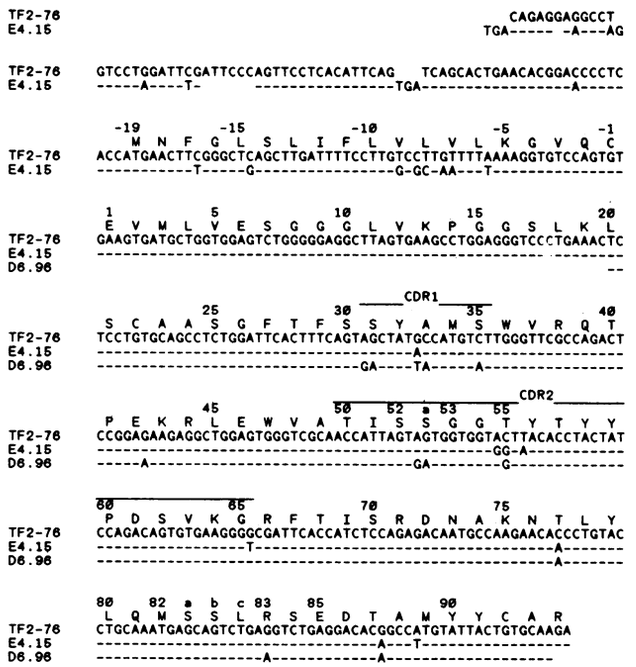

FIG. 1. Nucleotide sequence comparison of V_H 76 with V_H from two members of the 7183 V_H family (10). The sequence for the TF2-76 5′ untranslated signal and V_H region is from cDNA. The sequences for V_H E4.15 and V_H D6.96 are from genomic clones of rearranged *V* regions from lines 40E4 and 22D6. In comparing the V_Hs of TF2-76 and E4.15, we assumed a splice site between codons −5 (AAA) and −4 (GGT) in the E4.15 gene (10).

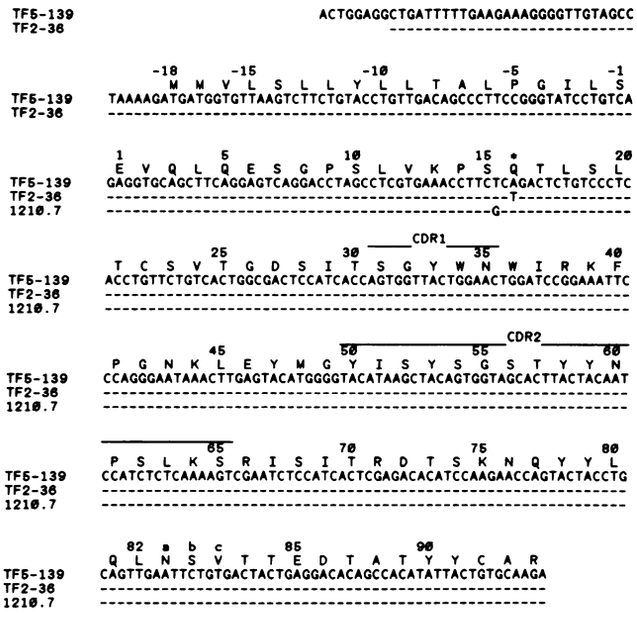

FIG. 2. Nucleotide sequences of V_H 36 from cDNA clone pμ36[21] and of V_H 139 from cDNA clone pα139[3]. The sequence for V_H 1210.7 was from a BALB/c germ-line genomic clone (30).

third *D* segment, for which a germ-line *D* segment cannot be identified. Both TF2-36 and TF2-76 antibodies utilize J_{H4} (32) but have recombined at different sites. Because the TF2-76 antibody also uses a different V_H, we cannot correlate DNP-binding with a particular V_H, *D*, or J_H segment.

The κ Light Chains from the TF2-36 and TF5-139 Antibodies Utilize Different V_κ Gene Segments. A cDNA clone (pκ36[2]) containing most of the TF2-36 V_κ gene segment (V_κ 36) was isolated and sequenced (Fig. 4). Since pκ36[2] does not contain the 5′ portion of the κ light chain mRNA, the N-terminal amino acid sequence of the κ light chain protein from the TF2-36 antibody was determined. DNP-binding IgM was purified from TF2-36 hybridoma grown in ascites, and the κ light chain protein was isolated by NaDodSO$_4$/PAGE. The amino acid sequence for the first 18 residues showed a major and minor species; for the major species, residues 1–9 are given in Fig. 4, and residues 10–18 match the translated sequence from pκ36[2]. When the nucleotide sequence of V_κ 36 was translated and compared with the amino acid sequences of other V_κ light chain regions, it showed closest homology with members of the $V_{\kappa 1}$ group (33, 34). The $V_{\kappa 1}$ group consensus sequence and the translated V_κ 36 gene sequence differed by one amino acid at codon 46. A partial N-terminal amino acid sequence through residue 54 has been determined

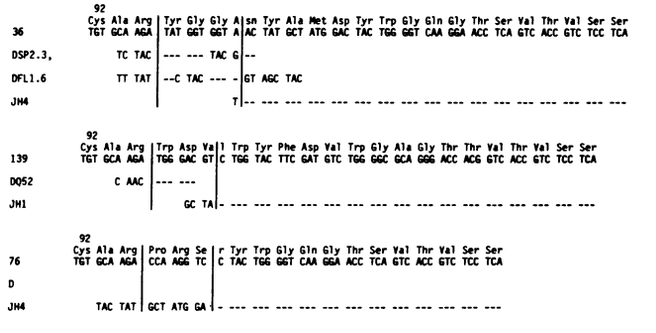

FIG. 3. Nucleotide sequences of the *D* and J_H gene segments used by TF2-36, TF5-139, and TF2-76 antibodies. The sequences for germ-line *D* segments (31) and germ-line J_H segments (32) are from BALB/c genomic clones.

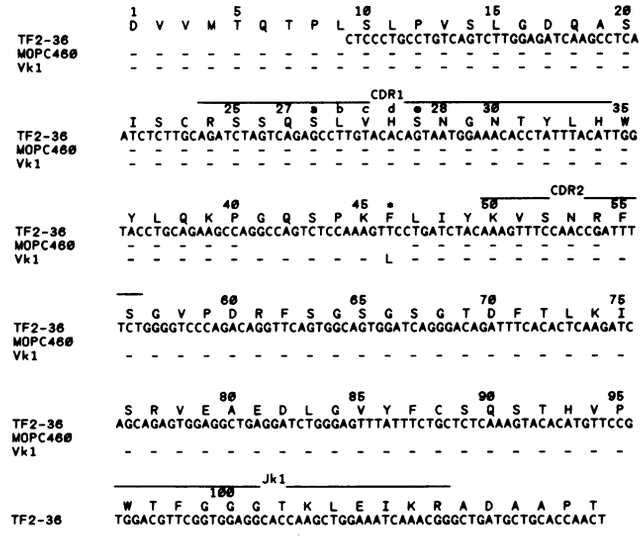

FIG. 4. Nucleotide and amino acid sequence of V_κ 36. Nucleotide sequence was from cDNA clone pκ36[2]. The first 18 amino acid residues were sequenced directly from the κ light chain isolated on NaDodSO$_4$/PAGE from affinity-purified IgM. The partial amino acid sequence for MOPC460 κ light chain is from ref. 33. The consensus amino acid sequence for the $V_{\kappa 1}$ subgroup was derived from seven κ light chains (33, 34). The sequence for the germ-line $J_{\kappa 1}$ segment (35, 36) is from BALB/c genomic clones.

for the MOPC460 (a DNP-binding myeloma) V_κ light chain region (35). For the residues sequenced, the MOPC460 and the TF2-36 κ light chains share complete homology.

A cDNA clone (pκ139[17]) containing all of the TF5-139 V_κ gene segment (V_κ 139) was isolated and sequenced. The translated sequence of V_κ 139 most closely resembles the amino acid sequence of the MCPC603 (a phosphocholine-binding myeloma) V_κ region (ref. 37; Fig. 5). The κ light chain cDNA clones from TF2-36 (pκ36[2]) and from TF5-139 (pκ139[17]) contain the $J_{\kappa 1}$ gene segment (refs. 35 and 36; Figs. 4 and 5).

SP2/0 poly(A)$^+$ RNA hybridizes with a cloned C_κ probe in a RNA blot (data not shown). A V_κ 36 probe was isolated as a 167-bp *Rsa* I fragment from pκ36[2] and was hybridized in a RNA blot with TF2-36 and SP2/0 poly(A)$^+$ RNA. Only RNA from TF2-36 hybridizes with this probe (data not shown), therefore, we assume that pκ36[2] is a cDNA clone of κ light chain mRNA expressed by the neonatal spleen cell. As described above, an N-terminal amino acid sequence of κ light chain isolated from affinity-purified IgM from TF2-36 contains a major and minor species. The presence of a minor species was indicated by two different amino acid residues at position 2; only one amino acid residue was detected at positions 1 and 3–18. Residues 10–18 of the major species matched the translated sequence from pκ36[2]. Since only one κ light chain protein is expressed by an individual spleen cell (38), the minor species was probably expressed by the early isolate of SP2/0 used to make these hybridomas. This κ light chain protein may participate in mixed IgM molecules that are isolated as a minor species in affinity-purified, DNP-binding IgM. Together, the RNA blot and N-terminal amino acid sequence data indicate that pκ36[2] contains the V_κ region used by the TF2-36 DNP-specific, neonatal hybridoma protein.

N-terminal amino acid sequence for the κ light chain isolated from DNP-binding IgA purified from TF5-139 grown in ascites matched the translated sequence from pκ139[17]. In summary, the neonatal DNP-specific proteins produced by hybridomas TF2-36 and TF5-139 utilize the same V_H and J_κ

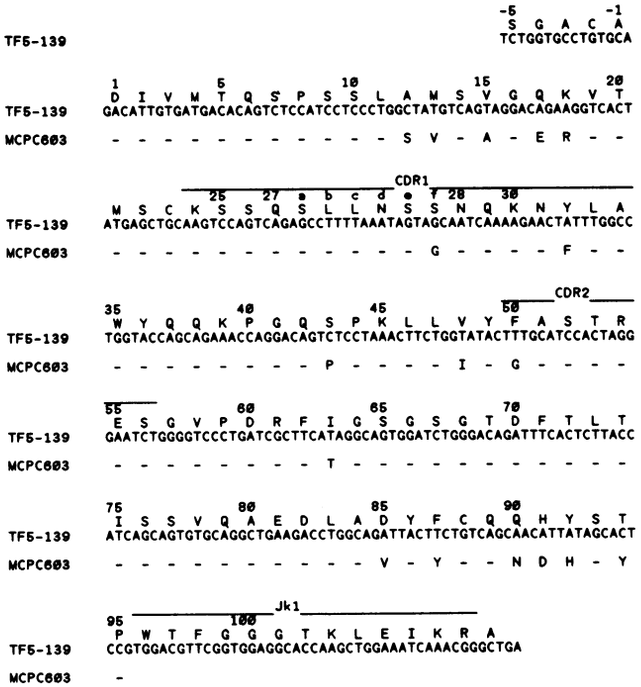

FIG. 5. Nucleotide sequence of V_κ 139 from clone pκ139[17] encoding the DNP-binding κ light chain. The amino acid sequence for V_κ 139 is compared with the amino acid sequence for MCPC603 (37). Amino acid sequence analysis of the first 18 residues of the κ light chain gave a perfect match with the deduced sequence.

segments but utilize different D, J_H, and V_κ segments (Table 1).

DISCUSSION

During the past two decades, a considerable amount of information has accumulated demonstrating the time-ordered acquisition of antibody responses during fetal and neonatal development (3–9, 39–41). In some cases the age at which responses to a given antigen first appear may be dependent upon the ability of the organism to process the antigen or present it to appropriate B cells. However, analyses of responses to influenza hemagglutinin, TNP, and DNP have demonstrated that, although neonatal B cells can respond to these antigens, a few identifiable clonotypes reproducibly appear earlier in development than the vast majority of clonotypes characteristic of the adult repertoire responsive to these antigens (3–6). For example, it has been calculated that adult BALB/c mice express between 10^3 and 10^4 distinct clonotypes responsive to DNP (1, 3). However, during early neonatal development, the response to DNP is dominated by very few clonotypes (3, 4).

Table 1. Gene segment utilization by neonatal anti-DNP hybridomas

Hybridoma	Isotype	Maximum idiotype representation*	H chain			L chain	
			V_H	D	J_H	V	J
TF2-36	μ, κ	Day 3	36-60	SP2.3/ FL16.1	4	MOPC460	1
TF2-76	μ, κ	Day 12	7183	NI	4	NI	NI
TF5-139	α, κ	Day 3	36-60	Q52	1	MCPC603	1

NI, not identified.
*Days after birth at which the proportion of idiotype-positive monoclonal antibody responses was highest.

Recently, a molecular basis for the preferential early expression of certain clonotypes has been suggested by the finding that V_H rearrangements in Ab-MuLV-transformed fetal liver pre-B-cells are skewed towards members of the 7183 V_H family. In particular, V_H 81X is rearranged in almost half of these cells (10). Since the 7183 V_H family maps closest to the J_H segments, Yancopoulos *et al.* (10) proposed that recombination frequency in fetal liver pre-B-cells is influenced by J_H proximity. Consistent with this conclusion, fetal liver hybridomas have been shown to exhibit the same skewing towards 7183 V_H family rearrangement (10, 11), and pre-B-cell and B-cell neonatal liver hybridomas contain a disproportionately high number of 7183 V_H family rearrangements (11). Neonatal liver hybridomas also exhibit rearrangements to other V_H families (11). In particular, the same number of neonatal hybridomas were found to contain functionally rearranged V_H from the Q52 and the J558 V_H families. However, since the J558 V_H family is at least 10 times larger than the Q52 family, the more J_H-proximal Q52 family rearranges at a disproportionately higher frequency than does the J558 V_H family. Consequently, Perlmutter *et al.* (11) have proposed that, early in neonatal development, H chain rearrangement continues to follow the order of V_H genes on the chromosome, even after the early dominance of the 7183 V_H family subsides.

Since B cells isolated from the spleen of newborn mice are representative of clones that continue to expand for the first few days of neonatal life (3), it would seem likely that clonotypes that dominate responses of 1- to 3-day-old neonates would reflect the specificities present in pre-B-cells found late in fetal development. In order to determine potential relationships between the order of V_H rearrangement found in Ab-MuLV-transformed fetal liver cells and the patterned appearance of antigen-responsive B cells in neonatal spleens, we have cloned and sequenced heavy and light chain V region cDNAs of three hybridoma antibodies whose idiotypes are predominant among neonatal B cells responsive to DNP; our results are summarized in Table 1. We found that one of the three hybridomas, TF2-76, uses a member of the 7183 V_H family. V_H 76 has 97% sequence homology with V_H E4.15, which was isolated from a V_H–D–J_H rearrangement that occurred during culture of an Ab-MuLV-transformed fetal liver pre-B-cell line (10). However, V_H 76 is utilized by an anti-DNP clonotype that is reproducibly expressed in high frequency during the second week after birth and is rare among monoclonal antibodies derived from spleen cells obtained during the first week after birth. The relatively late expression of this clonotype may indicate that the V_H 76 gene segment *per se* does not rearrange as early as other members of the 7183 V_H family. Alternatively, it is possible that rearrangement of V_H 76 occurs early in development but that rearrangement of V_κ 76 occurs relatively late in postnatal development.

The two DNP-responsive clonotypes that are predominant in 3-day-old neonates share idiotype with either TF2-36 or TF5-139. Both of these hybridomas utilize a member of the 36-60 V_H family. This V_H family has been tentatively mapped 5′ to the J558 V_H family (R. Riblet, personal communication) and, therefore, is quite distal to the 7183 V_H family and J_H. V_H 36 and V_H 139 differ by one nucleotide, and their signal peptide and 5′ untranslated region share complete identity (see Fig. 2). Therefore, TF2-36 and TF5-139 H chains probably utilize the same germ-line V_H. V_H 36 and V_H 139 share a silent nucleotide difference from the BALB/c germ-line V_H 1210.7 segment (see Fig. 2). We believe therefore that V_H 36 and V_H 139 are probably derived from V_H 1210.7. It is possible that BALB/c mice contain another V_H that differs from V_H 1210.7 by one nucleotide; however, it is more likely that the difference is due to genetic polymorphism between the sublines of BALB/c mice from which the V_H 1210.7 gene

was cloned and from which hybridomas TF2-36 and TF5-139 were obtained. It is unlikely that this difference reflects an identical somatic mutation, since hybridomas TF2-36 and TF5-139 were produced in two separate transfer fusions. If V_H 36 and V_H 139 are derived from a polymorphic form of V_H 1210.7, then V_H 139, which differs from V_H 1210.7 at a single base, might represent the germ-line V_H. The one nucleotide mismatch between V_H 36 and V_H 139 could be due to an error in reverse transcription (42) or to a somatic mutation that occurred in the B cell. It is of interest that hybridoma TF5-139, which might contain a germ-line V_H, is an IgA antibody, whereas TF2-36, an IgM antibody, may include a somatic mutation. Although somatic mutations are relatively rare in IgM antibodies, recent findings have indicated that somatic mutation may occur during B-cell development (43). Alternatively, a somatic mutation could have occurred after transfer of the neonatal B cells and immunization of the recipient with DNP-hemocyanin.

At day 3 after birth, the Id(TF2-36) is expressed on 15% of anti-DNP antibodies, and the Id(TF5-139) is expressed independently on 10% of anti-DNP antibodies. The idiotype assay used to identify these antibodies used adsorbed polyclonal rabbit anti-idiotype antisera raised against the hybridoma antibodies (4). Monoclonal antibodies were considered idiotype-positive only if their inhibition of the binding of the homologous antibody was equivalent (±20%) on a weight basis to the inhibition obtained by using the homologous antibody itself. Positivity in such assays generally implies sharing of most if not all epitopes. Thus, although the hybridomas TF2-36 and TF5-139 showed a low level of idiotype cross-reactivity (presumably due to their shared expression of V_H), they were clearly discriminated by this assay, as were monoclonal antibodies obtained from fragment cultures that were positive for either idiotype (N.R.K., unpublished observation). Therefore, since both V_H 36 and V_H 139 apparently utilize V_H 1210.7, at least 20–30% of anti-DNP-specific B cells at day 3 presumably express a rearranged V_H 1210.7 segment. Since one neonatal B cell in $3–5 \times 10^3$ respond to DNP-hemocyanin (3), then at least 1 per 10^4 neonatal B cells is both DNP-responsive and utilizes V_H 1210.7. The early expression of the Id(TF2-36) has recently been confirmed by J. Teale (44) who has found this idiotype on 10–30% of anti-DNP monoclonal antibodies obtained from cells derived from fetal liver organ cultures. Since V_H 1210.7 can associate with numerous D and J_H combinations and multiple light chains (this publication; ref. 32), it is likely that this V segment is utilized by a large proportion of the BALB/c neonatal antibodies.

The early expression of a member of the 36-60 V_H family, as compared with antibodies utilizing the 7183 V_H gene family in the neonatal anti-DNP response, may be an artifact resulting from our relatively small sample. Alternatively, this finding could indicate that utilization of V_H sequences in antigen-responsive B cells is not directly related to the frequency of V_H rearrangement. However, recent findings by Yancopoulos and Alt (personal communication) indicate that the 36-60 V_H family, like the 7183 V_H family, is expressed preferentially in fetal liver cells. Thus, the temporal appearance of clonotypes may indeed reflect preferential rearrangement of certain V segments; but the factors governing the frequency of rearrangement are likely to be more complex than proximity to J_H.

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