References and Notes

1. J. B. Dame *et al.*, *Science* **225**, 593 (1984).
2. V. Enea *et al.*, *ibid.*, p. 628.
3. C. Newbold, *Mol. Biochem. Parasitol.* **11**, 1 (1984).
4. A. A. Holder and R. R. Freeman, *J. Exp. Med.* **156**, 1528 (1982).
5. M. E. Perkins, *ibid.* **160**, 788 (1984); C. Sullivan and M. Perkins, unpublished observations.
6. H. Perlmann *et al.*, *J. Exp. Med.* **159**, 1686 (1984).
7. B. Wahlin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 7912 (1984); M. Wahlgren, in *Modern Approaches to Vaccines*, R. Lerner and R. Chanock, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., in press).
8. L. Villa-Komaroff *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3727 (1978); H. Okayama and P. Berg, *Mol. Cell Biol.* **2**, 161 (1982).
9. The cDNA library was constructed as described (*8*). Briefly, 2 μg of polyadenylated RNA from a late-stage *P. falciparum* schizont was primed with oligo(dT) and used to synthesize a cDNA strand with reverse transcriptase (*8*). The RNA template strand was replaced in a reaction that contained ribonuclease H, *E. coli* polymerase (Klenow fragment), and *E. coli* ligase as described (*8*). Blunt-end double-stranded cDNA molecules were generated with T4-DNA polymerase, and oligo(dC) tails (20 to 30 cytosines per end) were added with terminal deoxynucleotidyl transferase. These molecules were annealed to oligo(dG)-tailed pUC-9 made linear with Pst I [J. Viera and J. Messing, *Gene* **19**, 259 (1982); G. Heidecker and J. Messing, *Nucleic Acids Res.* **11**, 4891 (1983)] and were used to transform *E. coli* strain MC1061. Approximately 10^5 transformants were obtained and stored on sterile nitrocellulose filters (150 cm).
10. D. M. Helfman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 31 (1983); R. Young and R. Davis, *ibid.*, p. 1194.
11. JM103 [*Δ(lac-pro), thi, strA, supE, endA, sbcB, hsdR*, F′ *traD36, proAB, laci*^{q}, ZΔM15].
12. *Escherichia coli* strain JM103, transformed with individual cDNA clones in pUC-9, were grown from single colonies in Luria-Bertani broth at 37°C to an optical density at 560 nm of 0.1, induced with IPTG to a final concentration of 1 m*M*, and grown for an additional 4 to 6 hours. Cultures were subjected to centrifugation, and the bacteria were resuspended in one-tenth volume of SDS loading buffer, boiled for 2 minutes, and subjected to SDS–polyacrylamide gel electrophoresis.
13. L. S. Ozaki, P. Svec, R. S. Nussenzweig, V. Nussenzweig, G. N. Godson, *Cell* **34**, 815 (1983).
14. J. V. Ravetch, R. Feder, A. Pavlovec, G. Blobel, *Nature (London)* **312**, 616 (1984).
15. Cultures were grown as described (*12*), and the centrifugate was resuspended in one-tenth volumes of 50 m*M* tris (*p*H 8.0), 25 percent sucrose, 1 percent Nonidet P-40, 0.5 percent deoxycholate, 2 m*M* dithiothreitol, and 5 m*M* EDTA and sonicated for 20 seconds. Mice of the BALB/c strain were inoculated intraperitoneally and subcutaneously with 0.1 ml of extract in complete Freund's adjuvant and then 2 weeks later with an equal dose in incomplete Freund's adjuvant. A final dose was administered intravenously 1 week later. The mice were bled 1 week later.
16. M. Perkins and J. V. Ravetch, in preparation.
17. M. Perkins, unpublished observation.
18. E. J. Winchell, I. T. Ling, R. J. M. Wilson, *Mol. Biochem. Parasitol.* **10**, 297 (1984).
19. R. L. Coppel *et al.*, *Nature (London)* **306**, 751 (1983); *ibid.* **310**, 789 (1984); M. Koenen *et al.*, *ibid.* **311**, 382 (1984); R. L. Coppel *et al.*, *ibid.* **310**, 789 (1984).
20. G. N. Godson, J. Ellis, P. Svec, D. H. Schlesinger, V. Nussenzweig, *ibid.* **305**, 29 (1983).
21. F. Sanger, S. Nicklin, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977).
22. A. Maxam and W. Gilbert, *ibid.*, p. 560.
23. M. Wallach, *Mol. Biochem. Parisitol.* **6**, 335 (1982).

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Developmentally Controlled Expression of Immunoglobulin V_H Genes

Abstract. *Although antibody diversity arises mainly from apparently random combinatorial and somatic mutational mechanisms acting upon a limited number of germline antibody genes, the antibody repertoire develops in an ordered fashion during mammalian ontogeny. A series of early pre-B and B-lymphocyte cell lines were examined to determine whether an ordered rearrangement of gene families of the variable region of immunoglobulin heavy chains (V_H) may be the basis for the programmed development of the antibody response. The results indicated that the V_H repertoire of fetal B-lineage cells is largely restricted to the V_H 7183 gene family and that subsequent recruitment of additional V_H gene families occurs during neonatal development. These results have important implications in understanding the ontogeny of immune function.*

Analyses of genes encoding antibodies have resulted in the elucidation of the strategies that permit a limited number of sequences to direct the syntheses of millions of different antibody molecules (*1*). In particular, each antibody consists of heavy (H) and light (L) polypeptide chains that are encoded by multiple, discontinuous, germline gene segments that are juxtaposed in antibody-forming (B) cell precursors. Combinatorial joining of variable (V_H), diversity (D), and joining (J_H) gene segments (for heavy chains) and of V_L and J_L gene segments (for L chains), coupled with association of heavy and light polypeptides, can be calculated to yield more than 10^7 different antibody molecules from an estimated set of fewer than 10^3 gene segments (*2*). In addition, a process of localized somatic hypermutation introduces small deletions, insertions, and single base substitutions in and around rearranged antibody genes (*3*). In well-studied antibody populations, most of the observed sequence heterogeneity appears to result from the somatic hypermutation process acting upon rearrangements involving one or at most a few germline gene segments (*4*).

By analysis of available protein and nucleic acid sequence data it has been possible to group murine V_H gene segments into discrete families (*5*). Recombination studies suggest that the different murine V_H families are themselves grouped discretely and not interspersed and provide an order on murine chromosome 12 for the heavy chain locus: centromere–V_HJ558–V_HS107–V_HQ52–V_HM7183–D–J_H–C_μ, encompassing 2 to 3 map units (*6*). In most immune responses of restricted heterogeneity, antibodies of a given specificity use V_H gene segments derived from a single V_H family, although antibodies of many specificities may utilize members of the same V_H family (*7*).

Despite the randomness inherent in the mechanisms that propagate antibody variability, the development of the antibody repertoire follows a characteristic program (*8*). Antibody responses to some antigens, bacteriophage ϕX174 for example, appear during fetal life in both sheep (*9*) and mice (*10*), while responses to most protein antigens cannot be induced until some days after birth. Specific clones of antigen-responsive B cells appear at characteristic times during murine development (*11*). A comparable restriction in antibody diversity at early stages in development has also been described in the chicken (*12*), in the frog *Xenopus laevis* (*13*), and in humans (*14*).

Although complex mechanisms for immunoregulatory interactions between different cell types might explain the developmentally ordered acquisition of antibody specificities, there is considerable evidence that this is not the case; for example the early absence of B cells reactive with inulin (*15*) and α-(1 → 6) dextran (*8*) reflects an absolute lack of antigen-specific B cell precursors. In the mouse, B-lymphocyte precursors can be detected in the liver by day 12 of fetal life (*16*). Antibody gene rearrangement is developmentally ordered in these cells and begins with juxtaposition of D and J_H gene segments followed by V_H rearrangement to give a functional V_H–D–J_H–C_μ transcriptional unit (*17*). It continues with light-chain rearrangement (first κ and then, if necessary, λ) to produce a complete set of functional rearranged antibody genes, thus marking the differentiation of a B cell from a pre-B cell. Since the chronology of acquisition of antibody specificities reflects B cell development alone, and since B cell development is marked by an orderly process of gene rearrangements, we hypothesized that different V_H gene families might rearrange preferentially at different times during development.

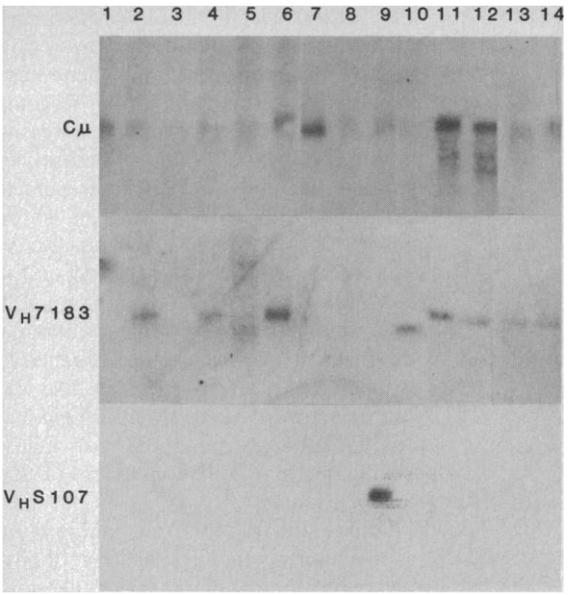

Fig. 1. V_H expression in fetal hybridomas. Total RNA was extracted from approximately 10^8 hybridoma cells by centrifugation in guanidinium thiocyanate and 10 μg of RNA was subjected to electrophoresis in formaldehyde-agarose gels (*28*). After transfer to nitrocellulose, sequences homologous to the indicated probes were detected by hybridization at 37°C in 0.8*M* sodium chloride, 0.1*M* tris, *p*H 7.5, 5× Denhardt's solution containing salmon sperm DNA (100 μg/ml) as carrier (*29*) and 10 percent dextran sulfate and 50 percent formamide. The hybridization probes for this experiment were generated by nick translation (*30*) of pBR322 or M13mp8 plasmid subclones of the sequences identified in Table 3 to a specific activity of 10^8 cpm/μg. The S107 (*4*), Q52 (*5*), V_H 7183 (*31*), and J558 (*5*) probes contained 24, 24, 51, and 51 base pairs of J_H coding region, respectively; however, these were not sufficient to detect J_H sequences alone under our hybridization conditions as demonstrated by test hybridizations with ^{32}P-labeled J_H probes (*4*). Blots were washed in 0.15*M* sodium chloride, 0.015*M* sodium citrate at 68°C for 3 hours prior to autoradiography (*28*). The samples were analyzed on a single gel; one gel was used for each probe. The order of samples corresponds to that in Table 1. VHM21 (*31*) is the representative of the V_H 7183 family used (*5*). The V_H J606 family probe was V_HGAC (*4*).

To examine the V_H repertoire of fetal and neonatal pre-B and B lymphocytes, we constructed a large number of hybridoma cell lines by fusing lymphocytes from livers of mice of defined age with the murine nonsecreting plasmacytoma Ag8653, which does not contain endogenous C_μ messenger RNA (mRNA) (*18*). We then extracted RNA from each of these cell lines and examined the distribution of expressed V_H sequences by hybridization (Northern blotting) with cloned V_H probes of known structure representing five of the eight V_H families (*5*). Stringent washing of these Northern blots enabled the assignment of the majority of the expressed V_H sequences to one of these five families of murine V_H gene segments (*5*).

Table 1. Characteristics of early B-cell hybridomas.

Hybridoma	Age* (days)	Strain	Cytoplasmic isotype
10-169-3	F18	C57BL	μ
FL26-13	F16-17	C57BL	μκ
26-30-1	F19	BALB	μλ
26-11-3	F19	BALB	μ
15-56-1	F16-19	BALB	μκ
16-67-4	F16-19	BALB	μ
17-3-5-10	F19	BALB	μ
10-74-5	F18	C57BL	μ
FD5-6	F19	$CB6F_1$	μκ
16-43-5	F16-19	BALB	μκ
FLFB6-2	F16-18	BALB	μ
FLCB6-10	F16-18	BALB	μ
16-165-3	F16-19	BALB	μκ
FLFC5-5	F16-18	BALB	μ
4-9-12-7	F16-17	C57BL	μ
SA2-2	N1	$SJAF_1$	μκ
NBID1-3	N1	$SJAF_1$	μκ
NBDC3-18	N1	$SJAF_1$	μ
NBBB4-13	N1	BALB	μ
NBCC4-14	N1	BALB	μκ
NBGA1-1	N1	BALB	μκ
NBBB1-5	N1	BALB	μκ
NBJC2-8	N1	BALB	μ
NBAB2-9	N1	BALB	μ
AA1-3	N1	SJA × BALB	μκ
NBAC3-5	N1	BALB	μκ
NBFC2-21	N1	BALB	μ
NBFC4-4	N1	BALB	μ
AC2-1	N1	SJA × BALB	μκ

*Age at fusion: F, fetal; N, neonatal.

The age, strain of origin, and cytoplasmic staining (*18*) characteristics of 29 cell lines which we examined for V_H expression are tabulated in Table 1. A total of 15 cell lines derived from nine separate fusions with fetal liver obtained between 16 and 19 days of gestation were examined; all but six of these are classified as pre-B cells in origin since they contained cytoplasmic H chains (μ) but not L chains (κ or λ). The 14 neonatal cell lines (including six pre-B cell lines) were derived from four independent fusion events using livers obtained during the first day of extra-uterine life. In addition, 23 cell lines were obtained with livers from 2-day-old mice as a source of B lymphocytes (Table 2). Comparison of sequences from fetal B cells with those from neonatal day 1 pre-B cells provides a direct measure of the frequency of specific V_H rearrangements since these cells do not express surface antibody and therefore cannot be exposed to selection by antigen or regulatory networks. The neonatal, day 1 and day 2 B-cell hybridomas provide a second level of comparison reflecting maturation of pre-B cells to B cells as well as maturation of the stem cell population which generates these cells.

The level of H chain mRNA, as observed in Northern blot hybridizations of total RNA from 14 fetal B-lineage hybridomas (Fig. 1) was variable but quite high, resembling that seen in plasmacytomas (*18*). Surprisingly, 10 of these 14 hybridomas, and an additional cell line (4-9-12-7) transcribed V_H gene segments derived from the V_H 7183 gene family. This result cannot reflect systematic bias in the pool of cell lines as two of four cell lines from C57BL6 mice generated during three separate fusions transcribed the V_H 7183 family as did seven out of ten BALB/c cell lines which were obtained from five separate fusions. Moreover, it is unlikely that the predominance of V_H

Table 2. V_H expression by day 2 neonatal BALB/c hybridomas.

Hybridoma	Cytoplasmic isotype	V_H
LB5-2	μκ	Unassigned
GA5-1	μκ	J558
DC1-1	μκ	J558
JD4-6	μκ	Q52
JC6-4	μκ	Q52 (weak)*
PB2-2	μκ	7183
CD2-1	μκ	J558
2DX	μκ	Q52
BA6-1	μκ	J558
FA4-5	μκ	7183
EC2-3	μκ	J558
JD6-2	μκ	7183
GC6-1	μκ	J558
LA1-9	μκ	J606
BA2-1	μκ	M21
FB2-2	μκ	Unassigned
GA6-4	μκ	Unassigned
EA2-8	μκ	Unassigned
AC2-6	μκ	S107
GC1-5	μκ	Q52
JD2-1	μκ	Unassigned
GC6-3	μκ	7183
MD6-12	μκ	Q52

*Only faint hybridization was detected.

7183–expressing hybridomas was due to external selection of these precursors since fetal pre-B hybridomas which lack the surface receptor also displayed V_H 7183 predominance (Table 3). One cell line (FD 5-6) transcribed a member of the V_H S107 gene family, while genes belonging to a V_H family or families not represented among our cloned probe sequences were transcribed in the remaining three cell lines. The intensities of hybridization of V_H 7183 relative to those of C_μ varied among different cell lines, suggesting that more than one V_H gene segment was represented in this population (Fig. 1, and unpublished observations).

Since a high percentage of adult B cells utilize members of many V_H gene families as compared to the limited heterogeneity shown by neonatal cells (*19*), we examined neonatal hybridoma cell lines to determine whether the preference for V_H 7183 rearrangements persists after birth. In the first day of life, pre-B and B hybridoma cell lines derived from murine liver did not predominantly express sequences related to the V_H 7183 probe (Fig. 2 and Table 3). Instead, mRNA homologous to the V_H J558 probe was found in 3 of 14 neonatal hybridomas including two pre-B cell lines (Fig. 2 and cell line AC2-1); none of the fetal hybridoma lines used this large V_H gene family. Three of the day 1 neonatal B-cell hybridomas used a member of the V_H 7183 gene family and a fourth cell line contained H chain RNA which hybridized with a probe derived from the V_H Q52 gene family. Half of the day 1 neonatal cell lines used V_H genes which could not be identified with any of our probes.

To further examine V_H gene expression, we analyzed 23 cell lines derived from BALB/c livers obtained at day 2 of neonatal life. The results (Table 3) verify that while the V_H 7183 gene family was transcribed by the majority of fetal B-lineage cell lines, only 22 percent of day 2 neonatal B cells have rearranged a member of this V_H gene family. In the neonatal day 2 B cell lines, there were a large number of rearrangements involving the V_H Q52 and the V_H J558 gene families. In addition, 21 percent of the cells transcribed a V_H region which did not cross-hybridize with our V_H probes.

More than 70 percent of fetal V_H rearrangements involved one or more members of the V_H 7183 gene family. This high frequency cannot reflect antigen exposure since many of the fetal hybridoma lines were derived from pre-B cells which do not express the L chain and which cannot, therefore, assemble a functional antigen binding site. We believe that our results are free of selection artifact because of the large number of cell lines examined, representing a large number of independent fusion experiments that involved mice from many different litters.

Table 3. V_H expression by pre-B and B hybridomas of fetal and neonatal mice.

Cell phenotype	V_H gene family					Unassigned	V_H 7183 (%)
	7183	Q52	S107	J558	J606		
Pre-B (μ^+L^-)							
Fetal (9)*	7	0	0	0	0	2	78
Neonatal d1 (6)	0	0	0	2	0	4	0
B (μ^+L^+)							
Fetal (6)	4	0	1	0	0	1	66
Neonatal d1 (8)	3	1	0	1	0	3	38
Neonatal d2 (23)	5	5	1	6	1	5	22

*Number of cell lines in each category are indicated in parentheses.

Although the parent cell line used to generate these hybridomas was derived from a plasmacytoma which expresses the M21 V_H gene, there is no evidence to suggest that this could affect our results, especially since the percentage of M21-positive cells differed so dramatically in fetal as compared to neonatal hybridomas. In parallel studies we have found almost complete correlation between the V_H family detected by Northern blot analysis and V_H idiotypes detected by monoclonal and heterologous polyclonal antibodies to V_H (*20*). Thus, the majority of these cells contained functionally rearranged heavy-chain genes. We conclude that fetal and neonatal lymphocyte stem cells show distinct preferences for V_H gene family rearrangements. Analyses of murine fetal pre-B cells transformed by Abelson murine leukemia virus and normal fetal pre-B cells have also revealed a predilection for rearrangement of members of the V_H 7183 gene family (*21*).

The presence of an S107 V_H family rearrangement in the fetal population (cell FD5-6) was surprising since this family is small, including four V_H gene segments of which only three are functional (*4*), and since one of these V_H gene segments predominates in the antibody response to phosphorylcholine (PC), a response which does not appear until day 5 to day 7 of neonatal life (*4, 8*). Nucleotide sequence studies (*22*) of the expressed V_H gene of the FD5-6 cell line (Fig. 3) revealed that this V_H gene was assembled from the germline J_H1 gene segment (*1*), a D segment related to $D_{SP2.7}$ (*23*) and the V1 V_H segment of the V_H S107 gene family (*4*). A single substitution in the D region represents either somatic mutation of the germline SP2.7 sequence (*23*) or a previously unidentified germline D segment (*4, 22*). The FD5-6 H chain mRNA therefore resembles in most respects BALB/c mRNA's encoding PC-binding H chains (*4*). In fact, the FD5-6 cell expresses the T15 V_H idiotypic marker although it does not bind PC. Thus, the delayed appearance of immune responsiveness to PC during

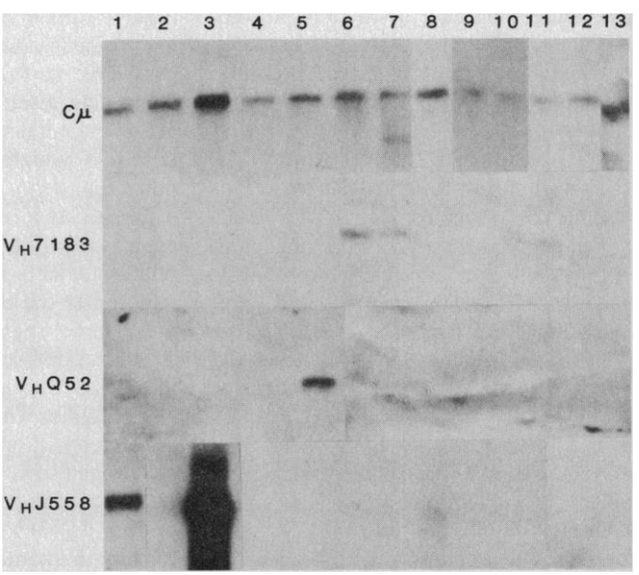

Fig. 2. V_H expression in day 1 neonatal hybridomas. Shown is a Northern blot analysis under conditions as specified as Fig. 1. The order of samples corresponds to Table 1 and the probes used are as described (*3, 7, 31*). Results from several Northern blots have been combined for each probe. Cell line AC2-1, which was positive with the V_H M21 probe, is not shown.

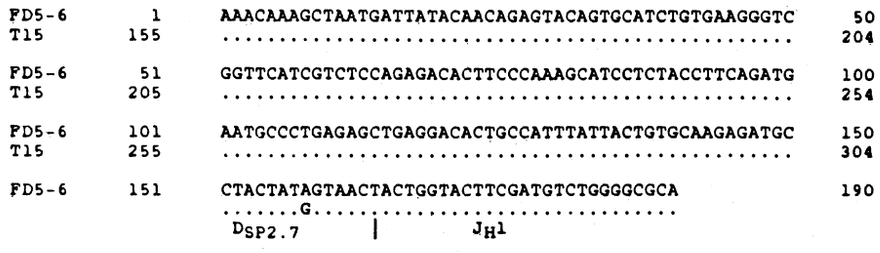

Fig. 3. Partial sequence of the H chain mRNA from fetal liver hybridoma FD5-6. Polyadenylated mRNA was isolated from total RNA by elution from oligo(dT) cellulose (*28*). Using a C_μ primer, the RNA sequence was determined directly by the dideoxynucleotide chain-termination method catalyzed by Avian myeloblastosis virus reverse transcriptase (*22*). Approximately 1 μg of template was used for each reaction and electrophoresis was performed using 100-cm 5 percent polyacrylamide gels. The sequence is compared with that of the germline BALB/c V1 gene segment T15 (*7*), the germline $D_{SP2.7}$ sequence (*23*), and the germline J_H1 sequence (*32*). Numbering of the T15 sequence begins at the first base of the mature secreted heavy chain, numbering of the FD5-6 sequence is arbitrary.

neonatal development is not the result of the lack of V1 (V_H S107) gene rearrangements.

The frequent rearrangements among neonatal B-cell hybridomas of genes belonging to the V_H Q52 and V_H J558 gene families (Table 3) are also of interest since genes within these families are associated with late-appearing α-(1 → 6)dextran and α-(1 → 3)dextran antibody responses, respectively (*8*). Thus V_H gene families associated with late-appearing B cells are rearranged during early neonatal development. Single genes within these families may be required to generate these antibodies and these particular genes may be late to rearrange. However, the expression of the specific gene associated with the late-appearing antibody response to PC by the fetal FD5-6 B hybridoma makes this idea less tenable. One explanation for the absence of certain antigen-specific precursors in young animals is that V_L rearrangement is restricted such that even though appropriate antigen-binding H chains may be assembled during fetal life, antigen-binding V_L regions are not produced until somewhat later in development. In support of this contention, isoelectric focusing analysis of the L chains in day 2 neonatal cell lines reveals only a few different spectrotypes while L chains from day 3 neonatal populations are more heterogeneous.

The pattern of V_H gene rearrangement of pre-B and B cell lines indicates that B-lineage cells which arise in utero predominantly rearrange genes within the V_H 7183 gene family. At or around the time of birth a change in rearrangement occurs, resulting in extensive rearrangement of other V_H gene families. This asymmetry in V_H gene expression may reflect a basic difference between the V_H 7183 gene family and all other V_H gene families. One explanation may be related to the proximity of this V_H gene family to the constant region genes (*6*). A specific chromosomal conformation in fetal B-lymphocyte progenitors may permit rearrangement of only the V_H gene family closest to C_μ. However, at or around the time of birth the H-chain locus may undergo a conformational transition in the B lymphocyte progenitor population which permits the rearrangement of other V_H gene families. Correlations between chromosomal order and ontogeny have been observed in other developing systems, for example the order of genes in the *Drosophila* bithorax complex parallels the anatomic order of the structures which these genes encode as well as the timing of development of these structures (*24*). The developmentally ordered expression of β-globin genes in mammalian erythropoiesis can be compared to the ordered rearrangement of antibody genes during lymphopoiesis. In both cases, the maturation of a self-renewing stem cell population results in alterations in gene expression. The chromosomal order of the β-globin genes predicts their order of expression in man, mouse, and rabbit (*25*), but not in goat (*26*). In this context, it would be interesting to analyze V_H expression in other mammalian species, though the genetic organization of the V_H locus has not been delineated as fully in other mammals as in mice. It would be interesting to identify mice with deletions or rearrangements in the V_H region which might alter the programmed read-out of these gene segments. Alternatively, a structural difference may exist between the V_H 7183 gene family and other V_H gene families which permits gene rearrangements of only the former by fetal B cell progenitors. Although the 3′ flanking sequence of the V_H 81X gene (a member of the V_H 7183 gene family) displays typical heptamer-nonamer sequences and 23-base-pair spacer signals (*1, 2, 21*), the V_H 7183 gene family may have other regulatory sequences that permit its rearrangement by fetal B-cell progenitors.

The timing of V_H rearrangement has interesting consequences for theories of acquistion of immunocompetence and tolerance. Immature B cells have increased sensitivity to tolerogenic doses of antigen and clonal deletion theories have been proposed to explain neonatal B-cell tolerance (*27*). Our results suggest that the majority of the B cell repertoire is generated after birth. Thus, mice cannot (in general) develop tolerance to antigens seen in utero on the basis of deletion of immunocompetent B cells, since few cells bearing V_H regions other than those from the 7183 V_H family are present prior to birth. This observation raises the possibility that V_H rearrangements are specifically limited in the fetal organism in order to minimize the development of an immune response to maternal or self antigens at a time when more complex regulatory mechanisms have not yet developed.

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References and Notes

1. P. Early and L. Hood, *Genet. Eng.* **3**, 157 (1981); T. Honjo, *Annu. Rev. Immunol.* **1**, 499 (1983).
2. P. Leder, *Hosp. Pract.* **18**, 73 (1983); S. Tonegawa, *Nature (London)* **302**, S75 (1983).
3. P. Gearhart and D. Bogenhagen, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3439 (1983); S. Kim, M. M. Davis, E. Sinn, P. Patten, L. Hood, *Cell* **27**, 573 (1981).
4. R. M. Perlmutter *et al.*, *Adv. Immunol.* **35**, 1 (1984); M. Siekevitz, S. Y. Huang, M. L. Gefter, *Eur. J. Immunol.* **13**, 123 (1983); A. L. M. Bothwell *et al.*, *Cell* **24**, 625 (1981); J. Rocca-Serera *et al.*, *EMBO J.* **2**, 867 (1983); R. M. Perlmutter *et al.*, *J. Exp. Med.* **159**, 179 (1984).
5. P. H. Brodeur and R. Riblet, *Eur. J. Immunol.*, in press; R. Dildrop, *Immunol. Today* **5**, 85 (1984).
6. P. H. Brodeur, M. A. Thompson, R. Riblet, in *Regulation of the Immune System*, E. Sercarz, H. Cantor, L. Chess, Eds. (Liss, New York, 1984); M. Weigert and R. Riblet, *Springer Semin. Immunopathol.* **1**, 133 (1978).
7. J. D. Capra and M. Fougereau, *Immunol. Today* **4**, 177 (1983).
8. C. Fernandez and G. Moller, *J. Exp. Med.* **147**, 645 (1978); J. Fung and H. Kohler, *ibid.* **152**, 1252 (1980); N. R. Klinman and J. L. Press, *Transplant Rev.* **24**, 41 (1975); N. H. Signal, P. J. Gearhart, J. L. Press, N. R. Klinman, *Nature (London)* **259**, 51 (1976); J. G. Howard and C. Hale, *Eur. J. Immunol.* **6**, 486 (1976).
9. A. M. Silverstein, J. W. Uhr, K. L. Kramer, R. J. Lukes, *J. Exp. Med.* **117**, 799 (1963).
10. W. Sherwin and D. T. Rowlands, Jr., *J. Immunol.* **113**, 1353 (1974).
11. K. A. Denis and N. R. Klinman, *J. Exp. Med.* **157**, 1170 (1983); N. R. Klinman and J. L. Press, *ibid.* **141**, 1133 (1975); M. P. Cancro, D. E.

Wylie, W. Gerhard, N. R. Klinman, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6577 (1979).
12. H. V. Huang and W. J. Dreyer, *J. Immunol.* **121**, 1738 (1978); S. Jalkanen, *Eur. J. Immunol.* **13**, 779 (1983).
13. L. Du Pasquier, B. Blomberg, C. C. A. Bernard, *Eur. J. Immunol.* **9**, 900 (1979).
14. R. T. Smith, D. V. Eitzman, M. E. Catlin, E. O. Wirtz, B. E. Miller, *Pediatrics* **33**, 163 (1964).
15. C. Bona, J. J. Mond, K. E. Stein, S. House, R. Lieberman, W. E. Paul, *J. Immunol.* **123**, 1484 (1979).
16. J. J. T. Owen *et al.*, *Eur. J. Immunol.* **5**, 468 (1975).
17. F. Alt, N. Rosenberg, S. Lewis, E. Thomas, D. Baltimore, *Cell* **27**, 38 (1981); R. L. Coffman and I. L. Weissman, *J. Mol. Cell. Immunol.* **1**, 31 (1983); R. Maki, J. Kearney, C. Paige, S. Tonegawa, *Science* **209**, 1366 (1980); F. W. Alt, G. D. Yancopoulos, T. K. Blackwell, C. Wood, E. Thomas, M. Ross, R. Coffman, N. Rosenberg, S. Tonegawa, D. Baltimore, *EMBO J.* **3**, 1209 (1984).
18. P. D. Burrows, M. Le Jeune, J. F. Kearney, *Nature (London)* **280**, 838 (1979); J. F. Kearney, A. Radbruch, B. Lisegang, K. Rajewsky, *J. Immunol.* **123**, 1548 (1979); R. P. Perry, D. E. Kelley, C. Coleclough, J. F. Kearney, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 247 (1981).
19. P. Basta, H. Kungawa, J. F. Kearney, D. E. Briles, *J. Immunol.* **130**, 2423 (1983).
20. J. F. Kearney, in preparation.
21. G. D. Yancopoulo *et al.*, *Nature (London)* **311**, 727 (1984); T. K. Blackwell, G. D. Yancopoulos, F. W. Alt, *Univ. Calif. Los Angeles Symp. Mol. Cell. Biol. N.S.*, in press.
22. G. M. Air, *Virology* **97**, 468 (1979); M. Kaartinen, G. Griffiths, A. F. Markham, C. Milstein, *Nature (London)* **304**, 320 (1983).
23. Y. Kurosawa and S. Tonegawa, *J. Exp. Med.* **155**, 201 (1982).
24. P. A. Lawrence and G. Morata, *Cell* **35**, 595 (1983).
25. A. Efstratiadis *et al.*, *ibid.* **21**, 653 (1980).
26. T. M. Townes, S. G. Shapiro, S. M. Wernke, J. B. Lingrel, *J. Biol. Chem.* **259**, 1896 (1984).
27. J. C. Cambier, J. R. Kettman, E. S. Vitteta, J. W. Uhr, *J. Exp. Med.* **144**, 293 (1976); N. R. Klinman, A. F. Scrater, D. H. Katz, *J. Immunol.* **126**, 1970 (1981).
28. E. Kraig *et al.*, *J. Exp. Med.* **158**, 192 (1983).
29. J. I. Mullins, W. J. Casey, M. O. Nicolson, N. Davidson, *Nucleic Acids Res.* **8**, 3287 (1980).
30. P. W. J. Rigby, M. Dieckmann, C. Rhodes, P. Berg, *J. Mol. Biol.* **113**, 237 (1977).
31. M. Kronenberg *et al.*, *J. Exp. Med.* **158**, 210 (1983); M. A. Thompson and M. P. Cancro, *J. Immunol.* **129**, 2372 (1982).
32. H. Sakano, R. Maki, Y. Kurosawa, R. Roeder, S. Tonegawa, *Nature (London)* **286**, 676 (1980).
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Plasma Homovanillic Acid Concentration and the Severity of Schizophrenic Illness

Abstract. *Concentrations of plasma homovanillic acid before treatment were highly correlated with global severity of illness in schizophrenic patients, both before and after treatment. In contrast, a fixed dose of haloperidol did not affect those concentrations. Thus, in patients with a diagnosis of schizophrenia, plasma homovanillic acid may reflect the severity of illness, but not be influenced by short-term pharmacological perturbations by neuroleptics.*

Plasma homovanillic acid (pHVA) of rodents and subhuman primates can reflect brain turnover of dopamine (*1, 2*). For example, neuroleptic-induced elevations in brain dopamine metabolism are reflected in changes in pHVA concentration (*3*). These and other results have led to the conclusion that about 50 percent of pHVA derives from the brain (*4*). Furthermore, pHVA may better reflect cortical dopaminergic activity than does cerebrospinal fluid (CSF) HVA concentration (*5*). Taken together, these data have encouraged the study of pHVA in humans.

Preliminary investigations of pHVA concentration have produced inconsistent results. Although pHVA concentration was decreased by the dopamine agonist apomorphine (*6*), a neuroleptic effect on pHVA concentration has not been shown. However, numerous methodological difficulties can influence the measurement of pHVA concentration. Activity and diet are likely to affect pHVA concentrations (*7*), and circadian rhythms are likely. Thus, human studies must control these factors to maximize whatever clinical utility pHVA concentration might have.

Dopaminergic mechanisms have been thought to play a role in the schizophrenias, primarily because neuroleptic agents all decrease dopaminergic neurotransmission (*8*). Conversely, drugs that enhance central dopaminergic activity can worsen schizophrenic symptoms (*9*). However, not all schizophrenics are benefited by neuroleptic treatment, nor are all worsened by dopaminergic agonists (*10*). More direct evidence for a dopaminergic abnormality in schizophrenia is lacking. To the extent that pHVA concentration reflects central dopaminergic mechanisms, pHVA can provide insight into the importance of dopamine in schizophrenia. We now report a positive relation between the severity of schizophrenic symptoms and concentrations of pHVA in drug-free patients.

Participating in the study were 18 schizophrenic males (mean age, 41 years) meeting Feighner or Research Diagnostic Criteria after a structured interview (*11*). All patients were on a standard low monoamine diet and free of any neuroleptics for a minimum of 4 weeks.

The study consisted of 29 consecutive days of haloperidol administration. On day 1 an indwelling catheter was inserted at 0830, and baseline samples for pHVA were drawn at 0930, 1000, 1030, and 1050. All patients were at complete bed rest for 12 hours before the study and had been fasting except for water for 14 hours. At 1100, haloperidol (0.2 mg/kg) was injected intramuscularly; beginning at 1110, seven additional samples for pHVA were drawn at hourly intervals (*12*). Thereafter, patients received 10 mg of haloperidol twice daily for 28 days. Assessment of symptom severity was performed on day 1 before the initial haloperidol administration and again on days 22 and 29. The assessment was performed by two independent raters using the Clinical General Impression (CGI) and Brief Psychiatric Rating Scale (BPRS) (*13*).

Haloperidol administration produced a sporadic effect on pHVA in individual patients, but no mean effect in all pa-

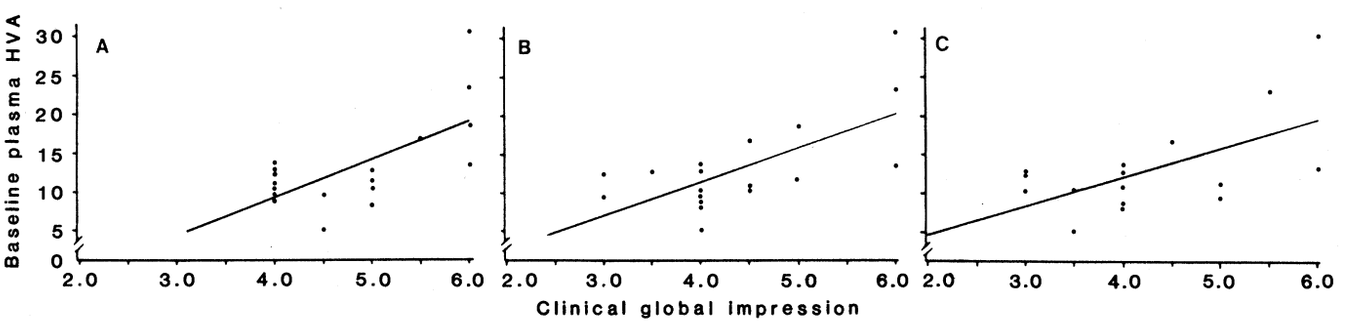

Fig. 1. Correlations between baseline pHVA and the CGI score on (A) day 1 (B) day 22, and (C) day 29.