Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 3839–3843, April 1995
Immunology

Invariance and restriction toward a limited set of self-antigens characterize neonatal IgM antibody repertoires and prevail in autoreactive repertoires of healthy adults

LUC MOUTHON*, ALBERTO NOBREGA†, NATHALIE NICOLAS†, SRINIVAS V. KAVERI*, CLAUDE BARREAU‡, ANTONIO COUTINHO†, AND MICHEL D. KAZATCHKINE*

*Institut National de la Santé et de la Recherche Médicale Unité 430 and Université Pierre et Marie Curie, Hôpital Broussais, 75014 Paris, France; †Unité d'Immunobiologie, Centre National de la Recherche Scientifique Unité de Recherche Affiliée 359, Institut Pasteur, 75015 Paris, France; and ‡Collection des Bactéries, Institut Pasteur, 75015 Paris, France

Communicated by K. Frank Austen, Brigham and Women's Hospital, Boston, MA, December 29, 1994

ABSTRACT **Analysis of the reactivity of IgM with self-antigens in tissues by a quantitative immunoblotting technique showed striking invariance among newborns in the human and in the mouse. The self-reactive repertoire of IgM of adults was also markedly conserved; it comprised most anti-self reactivities that prevailed among neonates. Multivariate analysis confirmed the homogeneity of IgM repertoires of neonates toward self- and non-self-antigens. Multivariate analysis discriminated between newborn and adult repertoires for reactivity with two of five sources of self-proteins and with non-self-antigens. Our observations support the concept that naturally activated B lymphocytes are selected early in development and throughout life for reactivity with a restricted set of self-antigens.**

The repertoire of natural antibodies in the serum of healthy individuals is the end result of successive selective processes. Differential rates of individual variable (V)-gene rearrangement, likely to reflect evolutionary selection, introduce a bias in the emergent bone marrow repertoires as compared with the germline (1–3). The resulting emergent bone marrow repertoire is continuously selected through the clonal elimination of some reactivities and preferential survival of others (4–6). Finally, there is evidence that repertoires of plasma cells and circulating antibodies in normal individuals differ from the repertoire of peripheral resting B lymphocytes (7, 8).

Autoantibody-associated heavy-chain V (V_H) genes and reactivities are preferentially expressed in fetal and perinatal B cells and antibodies (9–12). Autoreactive IgM and IgG also represent a major fraction of natural antibodies in the serum of healthy young adults (13, 14). Reactivity of natural antibodies with self-antigens has been documented by conventional immunochemical techniques using homologous and heterologous molecules as sources of antigens. The small number of antigens used in such studies does not allow a global analysis of the expressed self-reactive B-cell repertoire. We have recently developed a Western blot assay that allows a quantitative determination of hundreds of antibody reactivities toward antigens in homologous and syngeneic tissues (15). By using multiparametric statistical analysis, profiles of immunoreactivity in different strains of mice can be identified (16).

In the present study, we have used this approach to analyze the expressed IgM repertoire in umbilical cord blood and in the serum of healthy adults toward proteins in extracts of homologous tissues, bacteria, and plant that we selected as sources of self- and non-self-antigens. The results indicate that the human neonatal IgM antibody repertoire is invariant and directed toward a limited set of self-antigens. Invariance and restriction prevailed in autoreactive repertoires of healthy adults. Patterns of reactivity of IgM with non-self-antigens were poor in newborns and heterogeneous in adults. We interpret the data as indicating that natural IgM antibody repertoires are primarily driven by a limited subset of "internal" ligands which represent the targets for germline antibody specificities.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Sources of IgM. Cord blood was obtained from 15 healthy neonates born to healthy mothers, including 8 girls and 7 boys of Caucasian (n = 12) and African (n = 3) origin. Venous blood was collected from 18 healthy young men aged 20–40 years. Peripheral blood was also obtained from 1- and 8-week-old BALB/c and C57BL/6 mice (Iffa Credo). Sera were stored in aliquots at −20°C until use. Serum IgM concentration was measured by ELISA. IgM was purified by size-exclusion chromatography on Sephacryl HR S-300 (Pharmacia) from the serum of four adults and from Pentaglobin (Biotest Pharma, Dreieich, Germany), an IgM-enriched preparation of pooled human immunoglobulin. Purity of IgM was assessed by ELISA and SDS/PAGE.

Assessment of Antibody Repertoires by Quantitative Immunoblotting Analysis. To assess IgM antibody repertoires, we used a quantitative immunoblotting technique that allows the comparison of reactivities of antibodies from different sources with hundreds of antigens in tissue extracts (15). The sources of self-antigens were histologically normal human kidney, liver, thymus, stomach, and muscle obtained during surgical procedures and muscle, liver, lung, brain, thymus, and spleen obtained from normal CB20 adult mouse. The sources of non-self-antigens were *Pseudomonas aeruginosa* (C.I.P. A22); *Bacillus macquariensis* (C.I.P. 103269), a bacterium that has been reported only in Antarctica (17); *Kurthia sibirica* (C.I.P. 103418), a recently identified bacterium that was isolated from mammoth bowel (18); and *Bacopa amplexicaulis*, an aquatic plant originating from tropical areas. Proteins were extracted from tissues, bacteria, and plant by mechanical disruption and/or homogenization in 2% SDS/1.45 M 2-mercaptoethanol/125 mM Tris·HCl, pH 6.8, containing aprotinin (1 μg/ml), pepstatin (1 μg/ml), and EDTA (1 mM), on ice. Tissue samples were sonicated and boiled for 5 min at 100°C. Protein concentration was determined by Folin assay. Carbohydrates represented 5–10% (wt/wt) of the proteins in tissue extracts, as measured with phenol/sulfuric acid.

Proteins were subjected to preparative SDS/10% PAGE (19) at 20 mA per gel and transferred onto nitrocellulose (Schleicher & Schüll) for 60 min at 0.8 mA/cm^2 with a semi-dry electroblotter (model A; Ancos, Højby, Denmark). The membranes were blocked for 2 hr at room temperature with phosphate-buffered saline containing 0.2% Tween 20

Abbreviations: mAb, monoclonal antibody; PCA, principal-component analysis.

(20). Sera were incubated with the membranes after addition of one serum per slot in a cassette miniblot system (Immunetics, Cambridge, MA) with gentle rocking overnight at 4°C. The membranes were then washed with agitation in 0.1 M Tris/0.5 M NaCl/0.01 M glycine/0.1% Tween 20 before incubation with goat anti-human or anti-mouse IgM antibodies coupled to alkaline phosphatase (Southern Biotechnology Associates) for 2 hr at room temperature. Immunoreactivities were revealed with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate substrate (Promega). Quantitation of reactivities was performed by densitometry in the reflective mode with a high-resolution CCD camera (Masterscan; Scanalytics, Billerica, MA). Transferred proteins were then stained with a colloidal gold solution (Protogold, Biocell, Cardiff, U.K.). Lanes between immunoblots were subjected to a second densitometric analysis in order to characterize and quantitate transferred proteins. This allowed the immunoreactivity profiles to be compared by referring to their corresponding protein profile corrected for migration defects by superimposing corresponding protein peaks by computer. A reference human IgM preparation or a pool of normal mouse serum was included in each blot, in order to rescale the membranes transferred with a given protein extract and adjust for intensity of staining of different membranes. Data were analyzed with IGOR software (WaveMetrics, Lake Oswego, OR).

We have validated the method in terms of reproducibility and reliability for comparative analysis of different sources of immunoglobulin. Calculation of the areas under curve of the reactivity profiles of IgM with tissue extracts for serial dilutions of serum showed a dose-dependent decrease of immunoreactivity at IgM inputs of 5–50 μg/ml. The binding of IgM to the transferred extracts was saturable at concentrations of IgM above 50 μg/ml (data not shown). The reproducibility of the assay was assessed by comparing the areas under the curves of reactivity peaks with liver and muscle antigens in eight lanes of a membrane blotted with the same serum at one dilution. The 95% confidence interval was 30% of the mean area under the curve for peaks of immunoreactivity (data not shown). The anti-IgM antibodies used in our study recognized one band of 100 kDa in human and mouse tissue extracts and recognized no band in extracts of bacteria and plant. V-region dependence of the binding of IgM to antigens in tissue extracts was demonstrated as follows. (*i*) Patterns of reactivity of IgM with specific bands in the blots were selective in the case of mouse (15, 16) and human IgM. (*ii*) Soluble extracts of tissues dose-dependently inhibited the binding of serum IgM to the corresponding extract proteins immobilized on nitrocellulose. Inhibition by soluble extracts showed tissue specificity as well as different degrees of crossreactivity between various tissues. (*iii*) IgM monoclonal antibodies (mAbs) derived from spleen and bone marrow of normal adult unimmunized mice showed specific patterns of reactivity upon blotting with different tissue extracts: 60% of the mAbs did not react with any band, whereas each of the others showed a distinct profile of reactivity (21). (*iv*) The reactivity of an IgM anti-trinitrophenyl mAb mixed with IgM-containing supernatants of lipopolysaccharide-stimulated mouse B cells, with trinitrophenylated bovine serum albumin added to a bacterial extract, was inhibited by soluble trinitrophenylated bovine serum albumin (E. Malanchère and A.C., unpublished work).

Statistical Analysis. Reactivity profiles of IgM of newborns and adults with each set of antigens were divided into sections corresponding to defined peaks. To discriminate between groups of individuals, areas under curves were calculated in immunoblots of IgM of each individual with each source of antigens and submitted to principal-component analysis (PCA) (22). Separate PCA performed for reactivities of IgM of newborns and adults with each set of antigens allowed the calculation of respective variances of both groups of individuals. Multivariate statistical treatment of the data was performed with MATHEMATICA software (Wolfram Research, Champaign, IL).

RESULTS

Analysis of the Self-Reactive Repertoire of IgM in Cord Blood and in the Serum of Healthy Adult Donors. Reactivity of cord blood IgM of 15 neonates and serum IgM of 18 healthy young men with protein extracts of liver, muscle, stomach, thymus, and kidney was studied by immunoblotting. The IgM concentration was 0.064 ± 0.014 mg/ml (mean ± SD) and 1.17 ± 0.53 mg/ml in cord blood and in the serum of adults, respectively. Sera were diluted to an IgM concentration of 20 μg/ml. The amount of protein extract subjected to electrophoresis and transfer ranged between 100 and 600 μg per gel, depending on the tissue. All experiments were repeated twice.

The reactivity profiles of serum IgM of 15 neonates with kidney, liver, muscle, and thymus antigens are shown in Fig. 1. Only 8–10 protein bands among the multiple protein bands in the blots were recognized by serum IgM of each individual tested. The same protein bands were recognized by IgM of all individuals in each tissue tested. Some bands strongly stained with colloidal gold were not recognized by IgM, whereas others, present in low amounts, generated strong immunoreactivity (Fig. 2). There were only minor differences between individuals in the magnitude of the peaks of reactivity. No difference was observed between reactivity profiles obtained with either purified IgM or serum IgM, when tested at the same IgM concentration (data not shown). Together, these observations indicate that reactivities of serum IgM are directed toward a limited set of antigens present in homologous tissue extracts and that there is extensive similarity of the IgM self-reactive repertoire among human neonates.

The reactivity profile of neonatal serum IgM toward homologous proteins was then compared with that of IgM from young adults (Fig. 3). Six and 10 protein bands were recognized in the stomach extract by IgM of neonates and adults, respectively. The 4 major bands of neonatal IgM reactivity were included in the adult IgM reactivity patterns. Reactivity profiles of IgM of adult donors were largely conserved among individuals, although to a lesser extent than in the case of neonates. Some peaks of reactivity were specific to a given

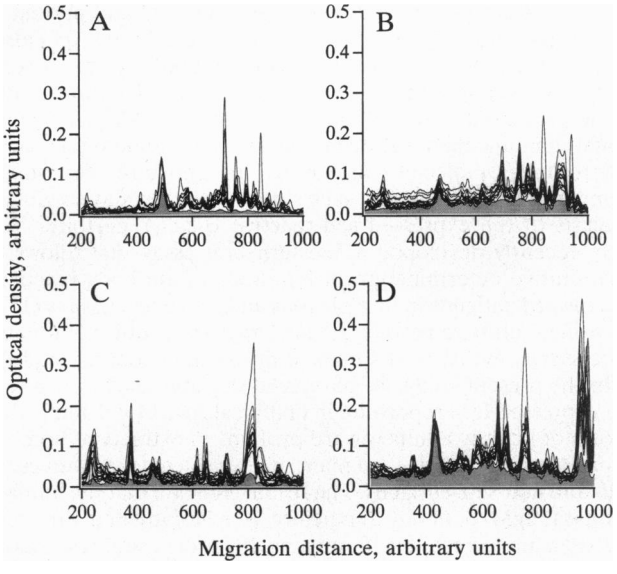

FIG. 1. Densitometric profiles of reactivity of cord blood IgM of 15 healthy neonates with normal human kidney (*A*), liver (*B*), muscle (*C*), and thymus (*D*) proteins. Sera were diluted to an IgM concentration of 20 μg/ml. Individual profiles are depicted as full lines. Shaded areas depict background obtained with anti-human IgM antibody alone.

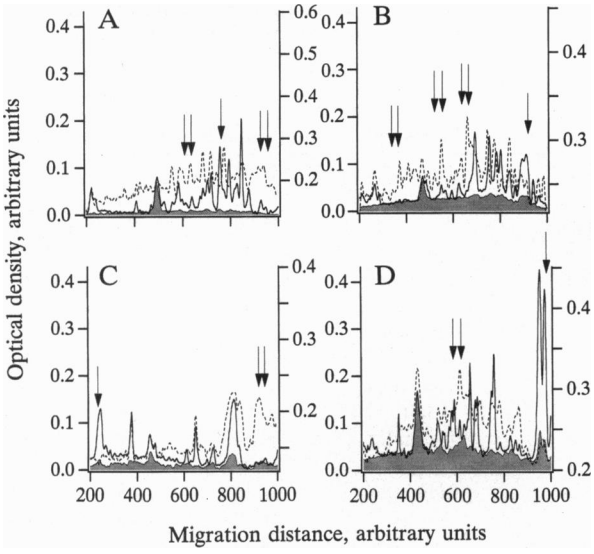

FIG. 2. Reactivity of cord blood IgM of a healthy neonate with kidney (*A*), liver (*B*), muscle (*C*), and thymus (*D*) proteins. Full lines depict the immunoreactive profiles (left axis). Broken lines depict densitometric quantitation of Protogold-stained proteins (right axis). Single arrows indicate bands associated with strong reactivity, although present in low amounts. Double arrows indicate bands strongly stained with Protogold and poorly or not recognized by IgM.

individual. There were inter-individual differences in the magnitude of the peaks of reactivity with a given protein band. Analysis of reactivity of serum IgM of adult donors with kidney and muscle extracts showed conserved patterns of reactivity (data not shown). In the case of liver and thymus antigens, profiles of reactivity of IgM exhibited some differences between donors regarding the bands recognized and magnitude of reactivity (data not shown). These results indicate that the adult serum IgM autoreactive repertoire is conserved among individuals and directed toward a limited set of self-antigens which includes those recognized by neonatal serum IgM.

Analysis of the Repertoire of IgM in Cord Blood and in Serum from Healthy Adults Toward Foreign Antigens. A small number of protein bands were recognized by IgM of neonates in extracts of *P. aeruginosa* and fewer bands in extracts of *B. macquariensis* and *K. sibirica* (Fig. 4). The same bands were recognized by IgM of all neonates tested, with minor inter-individual differences in the magnitude of the peaks of immunoreactivity. IgM from all adults that we studied showed reactivity with a large number of bands in extracts of *P. aeruginosa* and *B. macquariensis* and fewer bands in the extract of *K. sibirica* (Fig. 4). Inter-individual differences between patterns of reactivity of IgM of adult donors were striking in the case of extracts of *P. aeruginosa* and *B. macquariensis* and less pronounced in the case of extracts of *K. sibirica*. Patterns of reactivity of IgM of adults clearly differed from those of IgM of neonates in terms of number and magnitude of the peaks of

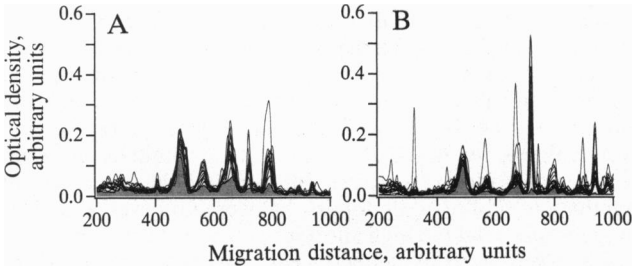

FIG. 3. Densitometric profiles of reactivity of cord blood IgM of 15 healthy neonates (*A*) and serum IgM of 18 healthy young men (*B*) with stomach proteins. Individual profiles are depicted as full lines.

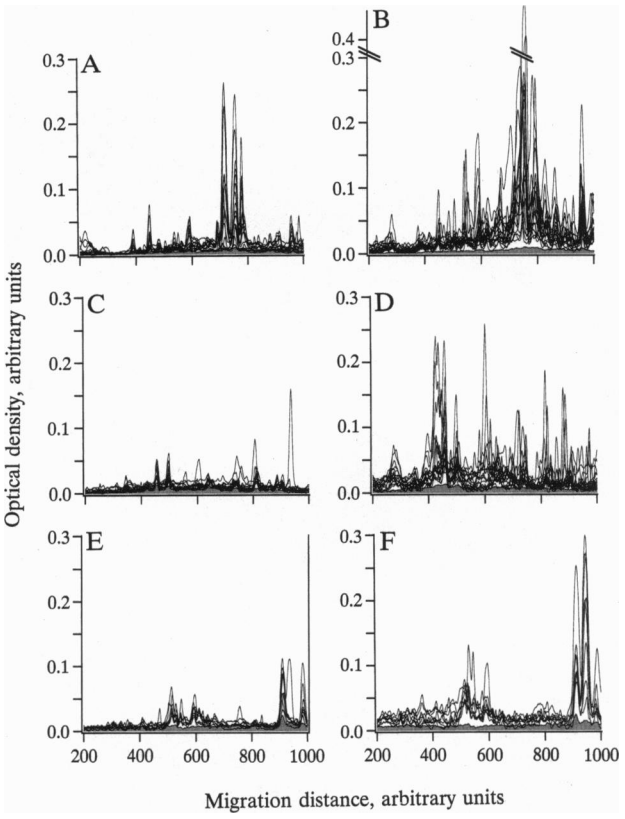

FIG. 4. Profiles of reactivity of IgM of 15 healthy neonates (*Left*) and serum IgM of 18 healthy young men (*Right*) with proteins from the bacteria *P. aeruginosa* (*A* and *B*), *B. macquariensis* (*C* and *D*), and *K. sibirica* (*E* and *F*). IgM was tested at 20 μg/ml. Individual reactivity profiles are depicted as full lines.

reactivity. We further investigated the reactivity of serum IgM with proteins of the tropical plant *Bacopa amplexicaulis*. Serum IgM of the 15 neonates reacted with a single band of apparent molecular mass of 60 kDa, whereas IgM of young adults recognized four to five bands, including that recognized by IgM of neonates (data not shown).

Comparative Analysis of Reactivity of Serum IgM from Neonates and Healthy Young Adults with Self- and Non-Self-Antigens by Multivariate Statistical Analysis. Reactivities of IgM of newborns and adults were quantitated by calculating the areas under curves delineated by sections assigned to each peak in the densitometric profile. The data were then submitted to PCA within a 30- to 39-dimension vector space, depending on the source of antigens, and fitted within the two-dimensional linear subspace (factors 1 and 2, accounting for 65–94% of the variance) that allowed the most powerful discrimination between individuals. Reactivities of IgM of newborns and adults with kidney, liver, and muscle proteins were not discriminated by PCA (Fig. 5). The repertoires of newborns and adults were distinct with regard to reactivities with stomach and thymus antigens (Fig. 5). Repertoires of reactivities of IgM were conserved among adults toward antigens of the kidney and thymus, although less than among neonates, as assessed by calculating the respective variances of reactivities of IgM of newborns and adults with these antigens (Table 1). There was heterogeneity among adults with regard to reactivities toward liver and stomach antigens; reactivities of IgM were conserved among adults and newborns in the case of muscle antigens (Table 1). PCA of the data obtained with each of the non-self-antigens discriminated between newborn and adult repertoires (Fig. 5). In addition, IgM repertoires of

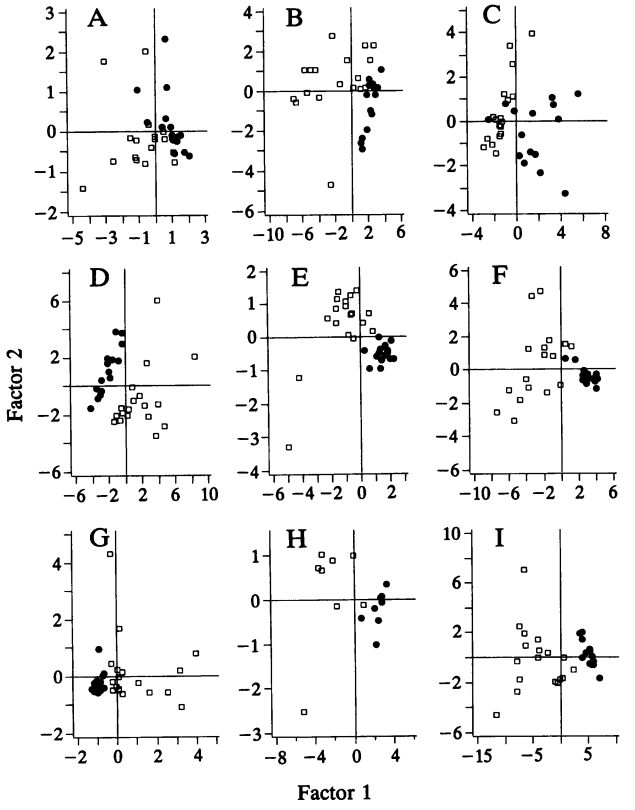

FIG. 5. PCA. Calculated areas under the curve corresponding to peaks of reactivity of IgM of neonates (●) and healthy young men (□) with self-antigens (*A–E*) and non-self-antigens (*F–I*) were subjected to PCA. The results are those obtained with IgM from the 15 neonates and 18 healthy men depicted in Figs. 1–4. Antigens were from kidney (*A*), liver (*B*), muscle (*C*), stomach (*D*), thymus (*E*), *P. aeruginosa* (*F*), *B. macquariensis* (*G*), *K. sibirica* (only eight neonates and eight adults were analyzed with *K. sibirica*) (*H*), and *Bacopa amplexicaulis* (*I*). Each individual is represented by a dot. The data relating to the 33 individuals were analyzed in a 30- to 39-dimension vector space, depending on the tissue extract, and fitted within a two-dimensional linear subspace (factors 1 and 2) corresponding to 65–94% of the variance.

newborn individuals clustered together for reactivities with non-self antigens, which was in contrast with the scattering of IgM repertoires of healthy adults (Fig. 5).

Analysis of the Self-Reactive Repertoire of Serum IgM of 8-Day-Old and Adult Mice. The self-reactive IgM repertoires of 8-day-old and adult BALB/c and C57BL/6 mice were compared by using six syngeneic organs as sources of antigens. The reactivity profiles of IgM in a pool of sera from six 1-week-old mice and of the mean profiles obtained with sera from five 8-week-old animals of the same strain toward muscle, liver, spleen, lung, brain, and thymus proteins expressed striking similarity (data not shown). These results further document the preservation in adult life of the original self-reactive repertoire expressed in the newborn.

DISCUSSION

Studies of autoreactive antibody repertoires have often utilized purified heterologous proteins as sources for self-antigens (9, 14). In the present work, we have used solubilized proteins from homologous tissue extracts to investigate the reactivity of IgM with a large panel of self-antigens. In addition to proteins, the carbohydrate or lipid moieties of glycoproteins and lipoproteins may have represented targets for some of the antibody reactivities that were scored. The differences observed between reactivity profiles of newborns and adults in

Table 1. Total variance of reactivities of IgM of newborns and adults with self- and non-self-antigens calculated by PCA

Tissue extract	Individuals	Total variance	Cumulative variance, % Factor 1	Cumulative variance, % Factor 2
Kidney	Neonates	29.52	57	86
	Adults	71.22	54	77
Liver	Neonates	45.70	80	86
	Adults	422.00	61	73
Muscle	Neonates	115.66	58	84
	Adults	97.01	58	78
Stomach	Neonates	97.59	63	86
	Adults	367.97	39	57
Thymus	Neonates	23.84	49	68
	Adults	60.53	44	58
P. aer.	Neonates	40.58	75	87
	Adults	280.91	50	70
B. mac.	Neonates	7.71	48	74
	Adults	121.00	45	65
K. sib.	Neonates	8.53	67	84
	Adults	48.91	64	82
Ba. amp.	Neonates	34.28	90	96
	Adults	431.68	59	84

Reactivities of IgM of newborns and of adults with antigens in extracts of five human tissues, of the bacteria *P. aeruginosa* (*P. aer.*), *B. macquariensis* (*B. mac.*), and *K. sibirica* (*K. sib.*), and of the plant *Bacopa amplexicaulis* (*Ba. amp.*) were analyzed separately in a 30- to 39-dimension vector space. Total and cumulative variances of factor 1 and 2 for reactivities of IgM of newborns and adults are shown.

the human may reflect, in part, reactivity with allotypic determinants. There are only a few examples of autoantibodies detected by reactivity with homologous antigens that were shown to recognize allotypic determinants in addition to self-determinants on target antigens (23). Homogeneity in reactivity profiles between individuals was also seen when mouse IgM reactivities with syngeneic tissue extracts were tested. Some of the epitopes identified by IgM may have been altered with respect to their conformation in the native protein. The latter considerations do not invalidate comparative analysis of reactivities of IgM from different sources.

Analysis of the reactivity of human cord blood IgM demonstrated that neonatal IgM recognized a small number of protein bands in human tissue extracts and that the pattern of reactivity was almost identical among individuals with regard to the bands recognized and the intensity of reactivity. The conserved character of reactivities of human cord blood IgM was established by low values of the calculated variance of reactivities of neonates with self- and non-self-antigens as compared with those of adults. Newborns included in the study were unrelated and belonged to various ethnic backgrounds. Reactivities of IgM of 8-day-old mice of different strains also showed extensive similarity. The results provide direct evidence that the plasma cell repertoire of newborns is restricted to reactivities toward a small set of self-antigens that is highly conserved between individuals.

Analysis of serum IgM of healthy adult donors also demonstrated restricted patterns of reactivity with self-antigens and conservation of these patterns among individuals. The reactivity profiles of adult IgM showed extensive similarity with those of newborn IgM in the case of kidney, muscle, and liver antigens for which PCA did not discriminate between newborn and adult repertoires. Inter-individual differences in reactivity of IgM of adult donors that were observed with liver antigens may relate to high allotypic polymorphism of proteins in this organ, even though we have restricted our analysis to male donors to avoid the complexity of the analysis of alloimmunized women. The self-reactive IgM repertoire of healthy men was distinct from that of newborns with regard to

reactivity with stomach and thymus antigens as assessed by PCA. Even in this case, more than half of the bands reactive with adult IgM in these two tissues were the same as those recognized by IgM of neonates.

Despite inter-individual differences in the intensity of reactivity with specific protein bands, the self-reactive repertoire of adult IgM appeared to be conserved. These results further extend our previous findings of a marked homogeneity among mice (16). Thus, the testing of mAbs and of supernatants of limiting-dilution cultures of B cells stimulated by a polyclonal mitogen has demonstrated the high discriminatory power of this method. Moreover, every mixture of up to 100,000 mitogen-activated B-cell clones is unique and readily distinguished from all others by a single gel (A.N., unpublished data; ref. 15). Conservation of serum reactivity patterns, therefore, reflects invariance in the repertoires of natural antibodies. Our observations strengthen the concept of a highly restricted and conserved naturally expressed self-reactive adult IgM antibody repertoire. The putative role of a limited set of target autoantigens in the establishment and maintenance of tolerance to self remains to be established (24).

Reactivity of cord blood IgM was restricted to ten bands in extracts from *P. aeruginosa*, four to five bands in *B. macquariensis* and *K. sibirica* extracts, and only one band in the case of *Bacopa amplexicaulis*. The same protein bands were recognized in a given tissue extract by IgM of all the neonates tested. Fewer bands were recognized in extracts of bacteria that are phylogenetically distant from commensal bacteria than in extracts of *P. aeruginosa*. The bands may represent proteins that are antigenically crossreactive with self-proteins. The paucity of reactivities of cord blood IgM with non-self-antigens strengthens the concept that the neonatal antibody repertoire is primarily self-reactive. In contrast to the patterns observed with cord blood IgM, a large number of reactivities were observed upon immunoblotting of serum IgM of adults with the extract of *P. aeruginosa*. Reactivity patterns with *P. aeruginosa* differed between individuals, contrasting with the homogeneous patterns of reactivity of adult IgM with self-antigens. Fewer bands of reactivity were observed, with proteins in extracts of *B. macquariensis* and *K. sibirica* displaying noticeable inter-individual heterogeneity among densitometric patterns. These observations are consistent with low selective pressure in the development of the adult antibody repertoire against foreign antigens.

The conserved character of the reactivities of cord blood IgM and their restriction to a limited set of self-antigens strongly suggest that the expressed neonatal B-cell repertoire is selected for recognition of self through V-region-dependent processes during fetal development (9–12). Alternatively, it could be argued that germline repertoires have been evolutionarily selected for reactivity toward a limited set of self-antigens. Preferential rearrangement and expression of a limited number of diversity (D)-region-proximal V_H genes have been demonstrated in the embryonic and perinatal murine repertoires (1–3), as well as a low frequency of terminal deoxynucleotidyltransferase-dependent N-sequence addition (25). Preferential D-region proximal V_H gene usage remains controversial, however, in studies of human fetal B-cell repertoires (11, 26–29). It has been demonstrated that immunoglobulin-dependent processes are required for the differentiation of precursor to mature B cells (30–32), and positive selection of B cells expressing a particular V_H gene has been shown in embryonic and perinatal life (5). Selection is more likely in the repertoire of serum IgM, since its production requires B-cell activation to plasma cells rather than simple survival. It is likely that the characteristics of the neonatal repertoires described here—i.e., marked restriction and invariance—result from the combination of evolutionarily selected genetic mechanisms favoring expression of a biased V-gene repertoire and further selection dependent on self-ligands.

That individuals of a given species share a restricted set of self-antigens as targets for a primordial B-cell repertoire is consistent with an "immunological homunculus" that would distortedly represent self and be instrumental in the establishment of tolerance to self (16, 33). Tolerance to self would be achieved through recognition of and positive selection by a conserved set of critical self-antigens by the immune system.

We are grateful to M. Haury and P. Ducimetière for advice and helpful suggestions, to E. Malanchère for providing some of the data relating to V-region dependency of IgM binding, and to Drs. J. H. Alexandre, P. Blot, C. Latremouille, and J. P. Sale for providing serum and tissue samples. This work was supported by the Institut National de la Santé et de la Recherche Médicale and Centre National de la Recherche Scientifique; The Central Laboratory of the Swiss Red Cross (Bern, Switzerland); and the Hyland Division of the Baxter Corporation (Glendale, CA). L.M. is a recipient of a grant from the Fondation pour la Recherche Médicale.

1. Yancopoulos, G. D., Desiderio, S. V., Paskind, M., Kearney, J. F., Baltimore, D. & Alt, F. W. (1984) *Nature (London)* **311,** 727–733.
2. Perlmutter, R. M., Kearney, J. F., Chang, S. P. & Hood, L. E. (1985) *Science* **227,** 1597–1601.
3. Yancopoulos, G. D., Maylinn, B. A. & Alt, F. W. (1988) *J. Exp. Med.* **168,** 417–435.
4. Gay, D., Saunders, T., Camper, S. & Weigert, M. (1993) *J. Exp. Med.* **177,** 999–1008.
5. Huetz, F., Carlsson, L., Tornberg, U. C. & Holmberg, D. (1993) *EMBO J.* **12,** 1819–1826.
6. Tiegs, S. L., Russel, M. D. & Namazee, D. (1993) *J. Exp. Med.* **177,** 1009–1020.
7. Portnoï, D., Freitas, A., Holmberg, D., Bandeira, A. & Coutinho, A. (1986) *J. Exp. Med.* **164,** 25–35.
8. Grandien, A., Modigliani, Y., Freitas, A., Andersson, J. & Coutinho, A. (1994) *Immunol. Rev.* **137,** 53–89.
9. Dighiero, G., Lymberi, P., Holmberg, D., Lundquist, I., Coutinho, A. & Avrameas, S. (1985) *J. Immunol.* **134,** 765–771.
10. Vakil, M. & Kearney, J. F. (1986) *Eur. J. Immunol.* **16,** 1151–1158.
11. Guigou, V., Guilbert, B., Moinier, D., Tonnelle, C., Boubli, L., Avrameas, S., Fougereau, M. & Fumoux, F. (1991) *J. Immunol.* **146,** 1368–1374.
12. Hillson, J. L., Opplinger, I. R., Sasso, E. H., Milner, E. C. B. & Wener, M. H. (1992) *J. Immunol.* **149,** 3741–3752.
13. Avrameas, S. (1991) *Immunol. Today* **12,** 154–159.
14. Hurez, V., Kaveri, S. V. & Kazatchkine, M. D. (1993) *Eur. J. Immunol.* **23,** 783–789.
15. Haury, M., Grandien, A., Sundblad, A., Coutinho, A. & Nobrega, A. (1994) *Scand. J. Immunol.* **39,** 79–87.
16. Nobrega, A., Haury, M., Grandien, A., Malanchère, E., Sunblad, A. & Coutinho, A. (1993) *Eur. J. Immunol.* **23,** 2851–2859.
17. Marshall, B. J. & Ohye, D. F. (1966) *J. Gen. Microbiol.* **44,** 41–46.
18. Belikova, V. A., Cheverach, N. V. & Kalakoutskii, L. V. (1986) *Microbiologia* **55,** 831–835.
19. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
20. Batteiger, B., Newhall, W. J. & Jones, R. B. (1982) *J. Immunol. Methods* **55,** 297–302.
21. Grandien, A., Fucs, R., Nobrega, A., Andersson, J. & Coutinho, A. (1994) *Eur. J. Immunol.* **24,** 1345–1352.
22. Jobson, J. D. (1992) *Applied Multivariate Data Analysis* (Springer, New York), Vol. 2.
23. Algiman, M., Dietrich, G., Nydegger, U., Boieldieu, D., Sultan, Y. & Kazatchkine, M. D. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 3795–3799.
24. Schwartz, R. S. & Stollar, B. D. (1994) *Immunol. Today* **15,** 27–32.
25. Feeney, A. J. (1990) *J. Exp. Med.* **172,** 1377–1390.
26. Jeong, H. D. & Teale, J. M. (1988) *J. Exp. Med.* **168,** 589–604.
27. Schroeder, H. W. & Wang, J. Y. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6146–6150.
28. Raaphorst, F. M., Timmers, E., Kenter, M. J. H., Tol, M. J. D. V., Vossen, J. M. & Schuurman, R. K. B. (1992) *Eur. J. Immunol.* **22,** 247–251.
29. Pascual, V., Verkruyse, L., Casey, M. L. & Capra, J. D. (1993) *J. Immunol.* **151,** 4164–4172.
30. Bosma, M. J. & Carol, A. M. (1991) *Annu. Rev. Immunol.* **9,** 323–350.
31. Kitamura, D., Roes, J., Kuhn, R. & Rajewsky, K. (1991) *Nature (London)* **350,** 423–426.
32. Jakobowitz, A., Vergara, G. J., Kennedy, J. L., Hales, J. F., McGuiness, R. P., Casentini-Borocz, D. E., Brenner, D. G. & Otten, G. R. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 2551–2555.
33. Cohen, I. R. (1992) *Immunol. Today* **13,** 490–494.