*Journal of Clinical Immunology, Vol. 14, No. 5, 1994*

# Isotypic Distribution of Anti-Pyruvate Dehydrogenase Antibodies in Patients with Primary Biliary Cirrhosis and Their Family Members

R. MARAN,[1,2] M. DUEYMES,[2] Y. ADLER,[1,2] Y. SHOENFELD,[1] and P. YOUINOU[2,3]

*Accepted: May 5, 1994*

IgG subclasses of anti-pyruvate dehydrogenase (PDH) antibodies were determined in 72 patients with primary biliary cirrhosis. All isotypes were detected, but IgG3, IgG1, and IgG2 predominated independently or in association. An average of 33.3 ± 19.1% of the anti-PDH IgG was IgG1 (mean optical density, 0.863 ± 0.783, vs 0.053 ± 0.038 in the normal controls), 25.0 ± 17.8 IgG2 (0.652 ± 0.656 vs 0.062 ± 0.030), 39.5 ± 23.4% IgG3 (1.140 ± 0.917 vs 0.010 ± 0.023), and 2.4 ± 7.4% IgG4 (0.060 ± 0.182 vs 0.012 ± 0.007). Anti-PDH IgG were restricted to IgG1 in the family members of patients (0.180 ± 0.403).

**KEY WORDS:** Primary biliary cirrhosis; anti-pyruvate deshydrogenase antibody; IgG subclasses.

## INTRODUCTION

Primary biliary cirrhosis (PBC) is a slowly progressive, but often fatal, liver disease (1). This is dominated by an inflammatory destruction of septal and interlobular bile ducts (2). A variety of immune abnormalities is thus consistent with an ongoing autoimmune process.

Given that they occur prominently in this condition, a particular class of antimitochondrial antibodies (AMA), collectively termed anti-M2 antibodies (3), serves as a reliable marker. These IgG autoantibodies target a multienzyme complex located on the inner mitochondrial membrane (4). In fact, most of the PBC sera react with the acetyltransferase (5) and/or the pyruvate-dehydrogenase (PDH) components of the group (6). Since indirect immunofluorescence (IFI) microscopy is influenced by observer interpretation, an enzyme-linked immunosorbent assay (ELISA) for detecting anti-PDH antibodies has recently been developed (7) and subsequently substantiated in PBC (6).

There is also compelling evidence that IgG antibody to certain antigens is restricted in its distribution between the subclasses (8, 9). Antibody isotype determination may therefore provide insight into the understanding of the thymus-dependent or thymus-independent type of response to the corresponding immunogen (Maran *et al.*, in press).

In the study presented here, we had the opportunity to evaluate a sizable group of unselected patients with PBC and some of their family members. Anti-PDH antibodies were analyzed using monoclonal antibodies (MAb) and found to belong not only to the IgG1 and IgG3 subclasses, but also to the IgG2 and IgG4 in the patients, but not in their relatives. Though not significantly, their profiles were more or less related to the clinical presentation of the disease, which suggests that the evolution of isotypic response may parallel that of the disorder.

## PATIENTS AND METHODS

### *Patients and Controls*

Seventy-two patients with PBC participated in the study. They all fulfilled the clinical, biochemical, and histological criteria for the disease (10). There were 63 women and 9 men, ranging in age from 15 to 80 years (mean 52.2 years). Routine laboratory testing was carried out and the results (serum bilirubine, alkaline phosphatase, and transaminases) were added to the database. Eighteen healthy first-degree relatives of seven of these patients were also inves-

[1]Research Unit of Autoimmune Diseases, Department of Medicine B, Chaïm Sheba Medical Center, Tel-Hashomer, Israel.
[2]Laboratory of Immunology, Brest University Medical School Hospital, BP 824, F29609 Brest Cedex, France.
[3]To whom correspondence should be addressed.



0271-9142/94/0900-0323$07.00/0 

tigated. Normal controls consisted of 20 subjects who were members of the clinical and laboratory staff or residents of an old people's home. They were approximately matched by age and sex with the patients.

### *Methods*

*Monoclonal Antibodies.* Mouse anti-human IgG1 (HP 6069), IgG2 (HP 6002 and HP 6014), IgG3 (HP 6047), and IgG4 (HP 6025) MAb were purchased from Zymed. Their affinity has been previously tested and their specificity demonstrated (11). In the IgG2 antibody tests, HP 6009 and HP 6014 were mixed 50:50, since the reactivity of anti-IgG2 MAb is exceedingly biased by the light-chain type.

*Antigen.* Purification of bovine heart PDH was performed using the technique described by Stanley and Perham (12). The enzyme preparation was dissolved in 50 n*M* 3-(*N*-morpholino)propanesulfonic acid, pH 7.0, supplemented with 3 m*M* ethylenediaminetetraacetic acid, 2 mM dithiothreitol, and 11% Triton X-100 to be stored at 4°C until required.

*Enzyme-Linked Immunosorbent Assay.* Microtiter plates (Nunc) were coated with 100 μl/ml of purified PDH. The incubation lasted 24 hr at 4°C and was followed by a saturation step with 0.2% phosphate-buffered saline (PBS) supplemented with bovine serum albumin and left 1 hr at room temperature (RT) and overnight at 4°C. Triplicate wells were incubated with patient serum diluted 1:200 in PBS supplemented with 0.05% Tween-20 and 1.5% gelatin (PBS-TG) for 30 min at RT. Following four washes in PBS-TG, antibody binding was detected with horseradish peroxidase (HRP)-conjugated $F(ab')_2$ anti-human IgG (Dakopatts) or mouse anti-human IgG subclass MAb. The latter were developed with a HRP-conjugated affinity-purified $F(ab')_2$ anti-mouse IgG. The optimal working dilutions for each MAb were defined as those giving comparable optical densities (OD) for approximately the same amounts of IgG subclasses. There were 1:2000 for anti-IgG1, 1:200 for anti-IgG2 HP 6002, 1:2000 for HP 6014, 1:5000 for anti-IgG3, and 1:1000 for anti-IgG4 MAb.

*Statistics.* The results were averaged and, for the five assays, OD greater than three standard deviations (SD) above the mean of the normal sera were considered positive ($n = 201$ for total IgG, $n = 20$ for IgG subclasses). The percentage of each subclass in the total OD sum of all four IgG subclass anti-PDH antibodies was calculated. All the results are arithmetic means and SD. Comparisons were made with the chi-square test with Yates' correction when required.

**Table I.** IgG Subclass Distribution of Anti-Pyruvate Dehydrogenase Antibodies in Patients with Primary Biliary Cirrhosis, Their Relatives, and Normal Controls[a]

| IgG subclass | No. of positive (percentage) | | |
|---|---|---|---|
| | Patients ($n = 72$) | Relatives ($n = 18$) | Controls ($n = 20$) |
| IgG1 | 61 (85) | 6 (33) | 1 (5) |
| IgG2 | 54 (75) | 0 (0) | 1 (5) |
| IgG3 | 58 (81) | 0 (0) | 1 (5) |
| IgG4 | 5 (7) | 0 (0) | 0 (0) |

[a]Optical densities greater than three standard deviations above the mean of the normal controls were considered positive.

## RESULTS

Of a total of 72 PBC patients screened, there were IgG antibodies to PDH in 66 (92%). As shown in Table I, IgG1 was found in 85, 33, and 5% of the patients, their relatives, and the controls, respectively (mean OD: 0.863 ± 0.783, 0.180 ± 0.403, and 0.056 ± 0.038); IgG2 in 75, 0, and 5% (0.652 ± 0.656, 0.048 ± 0.036, and 0.062 ± 0.030); IgG3 in 81, 0, and 5% (1.140 ± 0.917, 0.037 ± 0.041, and 0.010 ± 0.023); and IgG4 in 5, 0, and 0% (0.060 ± 0.182, 0.016 ± 0.006, and 0.012 ± 0.007). Various combinations were encountered in the patients, but not in their family members (Table II). The results were also expressed as the proportions of the IgG contributed to by each subclass. An average of 33.3 ± 19.1% was IgG1, 25.0 ± 17.8% IgG2, 39.5 ± 23.4% IgG3, and 2.4 ± 7.4% IgG4. Some patients, negative for IgG anti-PDH antibody in the global test, were positive

**Table II.** Anti-Pyruvate Dehydrogenase IgG Subclass Combinations in Patients with Primary Biliary Cirrhosis (PBC)[a]

| IgG subclass combination | Patients ($n = 72$) | Relatives ($n = 18$) | Controls ($n = 20$) |
|---|---|---|---|
| IgG1 | 3 | 6 | 0 |
| IgG1 + IgG2 | 5 | 0 | 0 |
| IgG1 + IgG3 | 8 | 0 | 0 |
| IgG1 + IgG2 + IgG3 | 40 | 0 | 1 |
| IgG1 + IgG2 + IgG3 + IgG4 | 5 | 0 | 0 |
| IgG2 | 1 | 2 | 0 |
| IgG2 + IgG3 | 3 | 0 | 0 |
| IgG3 | 2 | 0 | 0 |

[a]The IgG1 + IgG2 + IgG3 combination was significantly more frequent ($P < 0.01$) than the remaining combinations in the PBC patients.

**Table III.** Relation of Anti-Pyruvate Dehydrogenase (PDH) Antibodies to Laboratory Features in 63 Patients with Primary Biliary Cirrhosis

| Laboratory features | No. of positive (percentage) | | | |
|---|---|---|---|---|
| | IgG1 | IgG2 | IgG3 | IgG4 |
| Serum bilirubin (mg/L) | | | | |
| >2 ($n$ = 13) | 4 (31) | 8 (62) | 9 (69) | 1 (8) |
| 1.5–2 ($n$ = 4) | 1 (25) | 2 (50) | 1 (25) | 1 (25) |
| 1–1.5 ($n$ = 8) | 6 (75) | 5 (63) | 5 (63) | 0 (0) |
| <1 ($n$ = 38) | 26 (68) | 26 (68) | 28 (74) | 1 (3) |
| Alkaline phosphatase (U/L) | | | | |
| >200 ($n$ = 36) | 23 (64) | 21 (58) | 22 (61) | 2 (6) |
| 150–200 ($n$ = 11) | 8 (73) | 8 (73) | 9 (82) | 0 (0) |
| 120–150 ($n$ = 11) | 9 (82) | 7 (64) | 8 (73) | 1 (9) |
| <120 ($n$ = 5) | 2 (40) | 4 (80) | 3 (60) | 0 (0) |
| Transaminase (U/L) | | | | |
| >160 ($n$ = 11) | 6 (55) | 4 (36) | 3 (27) | 0 (0) |
| 120–160 ($n$ = 3) | 2 (67) | 2 (67) | 3 (100) | 0 (0) |
| 80–120 ($n$ = 13) | 8 (62) | 8 (62) | 11 (85) | 2 (15) |
| <80 ($n$ = 36) | 26 (72) | 26 (72) | 20 (72) | 1 (3) |

[a]The rank order of anti-PDH antibodies differed from one patient group to another, but this did not reach significance (chi-square test with Yates' correction).

for IgG1 or IgG1 plus IgG3 (three and four patients, respectively). Only two patients, negative for all the subclass-specific assays, were positive for the global test. The main biological characteristics were then examined according to the presence or absence of each IgG subclass antibody and the patient group was divided into four subgroups according to the level of serum bilirubin, alkaline phosphatase, and transaminases (Table III). The rank order of frequency was IgG3 (69% positive), IgG2 (62%), IgG1 (31%), and IgG4 (8%) in patients with the highest levels of serum bilirubin but not in the remainder; IgG1 (64%), IgG3 (61%), IgG2 (58%), and IgG4 (6%) in those with the highest levels of alkaline phosphatase but not in the remainder; and IgG1 (55%), IgG2 (36%), IgG3 (27%), and IgG4 (0%) in those with the highest levels of transaminases but not in the remainder. No significant differences could, however, be demonstrated between the patient subgroups.

## DISCUSSION

Quantitation of IgG subclasses used to be controversial, owing to different binding characteristics of the anti-isotype MAb required in the assay panel (13). The recent availability of thoroughly defined MAb allowed obviation of the technical problem (11). This is exemplified by the former discrepancy between the results obtained using the global test and the four IgG subclass assays. Our results tend to be more homogeneous, but some patients, negative for IgG anti-PDH antibody in the screening test, were positive for at least one of the IgG subclasses.

All isotypes of anti-PDH IgG could be detected, but IgG3, IgG1, and IgG2 (independently or in association) were produced predominantly in the PBC patients. Presentation of our results as a percentage value, rather than an OD level, makes it possible to evaluate the proportions of the IgG contributed to by each subclass. Again, the rank order was IgG3, IgG1, IgG2, and IgG4. We were not able to show a substantial difference between those with the highest levels of serum bilirubin, alkaline phosphatase, or transaminases and the remainder. These data are consistent with the results of Sundin (14), who confirmed the anti-PDH IgG subclass distribution by measuring the inhibitory capacity of sera on PDH enzyme, and those of Zhang *et al.* (15), who tested subclass-specific fractions in the AMA IIF assay.

A skewed pattern has also been found in a variety of autoantibodies, e.g., anti-Ro/SSA (8), anticardiolipin (16), antineutrophil cytoplasmic antigens (17), anti-denatured type II collagen (18), and antikeratin antibodies (19). Conversely, various autoantibodies, such as antibodies to double-stranded DNA (9), Sm (20), RNP (21), and acetylcholine receptor (22), were found to be restricted to IgG1 and IgG3 isotypes.

For many years, circumstantial evidence has been accumulating to indicate that autoantibody production is the result of polyclonal activation. However, there are also arguments to support the concept of antigen induction (23). As suggested by our PBC

data, chronic antigenic stimulation with protein antigen leads to progressively increased proportions of IgG2 and IgG4. Following the genomic arrangement of y3, y1, y2, and y4, a switch from predominantly IgG3 and IgG1 to predominantly IgG2 and IgG4 may thus occur.

In this respect, it is interesting that anti-PDH IgG were restricted to IgG1 in the family members of the patients and that IgG subclass combinations could not be found in these healthy relatives. However, the levels of IgG1 antibody were lower in relatives than in patients. Mitochondrial and other tissue antibodies have been described previously in relatives of patients with PBC (24), whereas a few instances of familial occurrence of the disease have thus far been reported (25). This raises the question of whether PBC is due to a putative environmental agent interacting with a genetically determined abnormal immune response.

## ACKNOWLEDGMENTS

We gratefully acknowledge Simone Forest for her excellent secretarial assistance.

## REFERENCES

1. James SP, Hoofnagle JH, Strober W, Jones EA: Primary biliary cirrhosis, a model autoimmune disease. Ann Intern Med 99:512–550, 1983
2. Dickson ER, Fleming CR, Ludwig J: Primary biliary cirrhosis. Progr Liver Dis 6:487–502, 1979
3. Baum H, Berg PA: The complex nature of mitochondrial antibodies and their relation to primary biliary cirrhosis. Semin Liver Dis 1:309–321, 1981
4. Gershwin ME, Mackay IR, Sturgess A, *et al.*: Identification and specificity of cDNA encoding the 70 Kd mitochondrial antigen recognized in primary biliary cirrhosis. J Immunol 138:3525–3531, 1988
5. Tuaillon N, André C, Briand JP, Pennec E, Muller S: A lipoyl synthetic octadecapeptide of dihydrolipoamide acetyltransferase specifically recognized by anti-M2 autoantibodies in primary biliary cirrhosis. J Immunol 148:445–450, 1992
6. Zurgil N, Bakimer R, Kaplan M, Youinou P, Shoenfeld Y: Anti-pyruvate deshydrogenase autoantibodies in primary biliary cirrhosis. J Clin Immunol 11:239–245, 1991
7. Zurgil N, Konikoff F, Bakimer R, Slor H, Shoenfeld Y: Detection of antimitochondrial antibodies. Characterization by enzyme immunoassay and immunoblotting. Autoimmunity 4:289–297, 1989
8. Maran R, Dueymes M, Pennec YL, Casburn-Budd R, Shoenfeld Y, Youinou P: Predominance of IgG1 subclass of anti-Ro/SSA, but not anti-La/SSB antibodies in primary Sjögren's syndrome. J Autoimmun 6:379–387, 1993
9. Dueymes M, Barrier J, Besancenot JF, *et al.*: Relationship in interleukin-4 to isotypic distribution of anti-double stranded DNA antibodies in systemic lupus erythematosus. Int Arch Allergy Immunol 101:408–415, 1993
10. Leevy CM, Popper H, Sherlock S: Diseases of the Liver and Biliary Tract. Standardization and Nomenclature, Diagnostic Criteria and Diagnostic Methodology. Chicago, Yearbook Medical, 1976
11. Jefferis R, Reimer CB, Skvaril F, *et al.*: Evaluation of monoclonal antibodies having specificity for human IgG subclasses. Results of an IUIS/WHO collaborative study. Immunol Lett 10:223–252, 1985
12. Stanley CJ, Perham RN: Purification of 2-oxo-acid dehydrogenase multienzyme complexes from ox heart by a new method. Biochem J 1991:147–150, 1980
13. Aucouturier P, Mounir S, Preud'homme JL: Distribution of IgG subclass levels in normal adult sera as determined by a competitive enzyme immunoassay using monoclonal antibodies. Diag Immunol 3:191–196, 1985
14. Sundin U: Antibody binding and inhibition of pyruvate deshydrogenase in sera from patients with primary biliary cirrhosis. Clin Exp Immunol 81:238–243, 1991
15. Zhang L, Weetman AP, Jayne DRW, *et al.*: Anti-mitochrondrial antibody IgG subclass distribution and affinity in primary biliary cirrhosis. Clin Exp Immunol 88:56–61, 1992
16. Gharavi AE, Harris EN, Lockslin MD, Hughes GRV, Elkon KB: IgG subclass and light chain distribution of anticardiolipin and anti-DNA antibodies in systemic lupus erythematosus. Ann Rheum Dis 47:286–290, 1988
17. Jayne DRW, Weetman AP, Lockwood CM: IgG subclass distribution of autoantibodies to neutrophil cytoplasmic antigens in systemic vasculitis. Clin Exp Immunol 84:476–481, 1991
18. Collins I, Morgan K, Clague RB, Brenchley PEC, Holt PJL: IgG subclass distribution of antinative type II collagen and antidenatured type II collagen antibodies in patients with rheumatoid arthritis. J Rheumatol 88:56–61, 1988
19. Vincent C, Serre G, Basile JP, *et al.*: Subclass distribution of IgG antibodies to the rat oesophagus stratum corneum (so-called anti-keratin antibodies) in rheumatoid arthritis. Clin Exp Immunol 81:83–89, 1990
20. Eisenberg RA, Dyer K, Cravens S, Fuller CR, Yount WJ: Subcall restriction and polyclonality of the lupus erythematosus marker antibody anti-Sm. J Clin Invest 75:1270–1277, 1985
21. Rubin RI, Tan FI, Chan EKI, Pollard KM, Tsay G, Tan EM: IgG subclasses of autoantibodies in systemic lupus erythematosus, Sjögren's syndrome and drug-induced autoimmunity. J Immunol 137:2528–2535, 1986
22. Nielsen FC, Rodgaard A, Djurup R: A triple antibody assay for the quantitation of plasma IgG subclass antibodies to acetylcholine receptors in patients with myasthenia gravis. J Immunol Methods 83:249–258, 1985
23. Roitt IM, Hutchings PR, Dawe KI, Sumar N, Bodman KB, Cooke A: The forces driving autoimmune disease. J Autoimmunity 5 (Suppl A):11–26, 1992
24. Gallbright RM, Smith M, MacKenzie RM, *et al.*: High prevalence of seroimmunologic abnormalities in relatives of patients with chronic active hepatitis or primary biliary cirrhosis. N Engl J Med 290:63–69, 1974
25. Jaup BH, Zettergren LSW: Familial occurrence of primary biliary cirrhosis associated with hypergammaglobulinemia in descendants. A family study. Gastroenterology 78:549–555, 1980