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J L Pasquali, … , J H Vaughan, D A Carson

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**Research Article**

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# Inheritance of Immunoglobulin M Rheumatoid-Factor Idiotypes

JEAN-LOUIS PASQUALI, SHERMAN FONG, CONSTANTINE TSOUKAS, JOHN H. VAUGHAN, and DENNIS A. CARSON, *Department of Clinical Research, Scripps Clinic and Research Foundation, La Jolla, California 92037*

ABSTRACT The idiotypic determinants on IgM rheumatoid factor (RF) from a single family have been analyzed. Rabbit Fab'2 antiidiotypic antibody was prepared against purified IgM-RF from a patient with rheumatoid arthritis. As measured by radioimmunoassay, the antiidiotype reacted with at least 90% of the patient's RF, but not with non-RF immunoglobulins from the same serum, nor with 10 of 11 polyclonal and monoclonal RF from unrelated individuals. Cross-reacting idiotypes were detected on RF in four of the patients' first degree relatives, spanning three generations, without apparent relation to HLA type or clinical rheumatoid arthritis. These results suggest that IgM-RF associated idiotypes were inherited in this family.

## INTRODUCTION

In experimental animals, the individual antigenic specificities, or idiotypes on certain induced antibodies against defined antigens are inherited (1–5). The inheritance of human idiotypes has been difficult to establish because of the need to do family studies in an outbred population, ethical considerations associated with deliberate immunization, and the marked heterogeneity of most conventional antibodies.

To approach this problem, we therefore decided to analyze the idiotypes on a naturally occurring autoantibody, IgM rheumatoid factor (RF),[1] found in patients with rheumatoid arthritis (RA). The first degree relatives of RA patients have an increased incidence of RF (6). These antibodies have restricted light-chain heterogeneity, and sometimes share cross-reactive idiotypes (7, 8).

We studied the idiotypes on IgM-RF from a classical RA patient, and four first degree relatives spanning three generations, two of whom had RA. Rabbit Fab'2 antiidiotypic antibodies, raised against purified RF from the proband and rendered individually specific by immunoadsorption, cross-reacted with RF from all four family members, but not with those of unrelated individuals with RA. The inheritance of the RF-associated idiotypes did not appear to be linked to the products of HLA A, B, and C histocompatibility loci.

## METHODS

*Patient and family.* The patient (Vi) is a 50-yr-old female with seropositive classical RA of 10 yr duration. Two of the patient's brothers died from myocardial infarctions; one was known to have RA. The sera of four first degree relatives were available. The patient's 79-yr-old mother (Mo) suffers from senility, mild cardiac insufficiency, and has suffered from definite seropositive RA for 18 yr. The patient's 60-yr-old sister (Si) has had mild definite RA for 15 yr. The patient's 24-yr-old daughter (Da) and 26-yr-old son (So) are both free of articular, hepatic, or infectious disease. All the sera were tested by latex titration (Hyland Diagnostics Div., Travenol Laboratories, Inc., Costa Mesa, Calif.) and gave positive results from 1/512 (Vi, Mo) to 1/128 (Da, So). Vi's husband's serum was tested by latex titration and gave a negative result.

*Purification of the patient's IgM-RF and IgM without RF activity.* Vi serum (50 ml) was treated with 40% saturated ammonium sulfate at 4°C, then centrifuged at 30,000 *g* for 30 min. The resulting globulins were dialyzed against isotonic phosphate-buffered saline (PBS), pH 7.4, then fractionated on a DEAE-52 (Whatman, Inc., Clifton, N. J.) chromatography column (60 ml), eluted with a stepwise NaCl gradient in 10 mM potassium phosphate, pH 8.0. The IgM fraction was identified by immunodiffusion, concentrated by pervaporation to 10 mg/ml in PBS, and absorbed onto 12 ml of cyanogen bromide-activated Sepharose 4B coupled with 300 mg of human Cohn fraction II (Sigma Chemical Co., St. Louis, Mo.) (9). After recirculating through the column at room temperature for 4 h and at 4°C for 12 h, which removed all the RF in the patient's IgM fraction (5 mg/ml) as assayed by radioimmunoassay (RIA), the RF-free IgM was stored at −20°C. The column was then washed with PBS and the RF eluted with a 0.1 M glycine-0.1 M HCl gradient. After neutralization with 0.5 M

Dr. Pasquali is supported by the French Foreign Ministry. His present address is Clinique Medicale A, Centre Hospitalo-Universitaire Strasbourg, France. Dr. Tsoukas is a fellow of the Arthritis Foundation.

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[1] *Abbreviations used in this paper*: anti-id, anti-idiotypic antibodies; PBS, phosphate buffered saline; RA, rheumatoid arthritis; RF, rheumatoid factor; RIA, radioimmunoassay; Vi, patient with RA.

potassium bicarbonate and dialysis against PBS, the IgM-RF fraction (1 mg/ml; 9 ml) was passed through a 2-ml column containing protein A (1 mg/ml) coupled to Sepharose 4B to remove most contaminating IgG. The IgM-RF fraction contained <1% IgG when analyzed by RIA.

*Preparation of Antiidiotypic Antibodies (Anti-id).* An adult rabbit (4 kg) from the Scripps Clinic animal stock was injected subcutaneously with 0.5 mg Vi IgM-RF in 1 ml complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) at 3-wk intervals. The rabbit was bled 10 d after the third injection.

The serum from this bleeding was treated with 40% ammonium sulfate at 4°C, centrifuged at 30,000 *g* for 30 min, dialyzed against PBS, then digested with 1% (wt/wt) pepsin (Boehringer-Mannheim Biochemicals, Indianapolis, Ind.) for 24 h at 37°C, in 0.1 M acetate buffer pH 4.1, 0.5 M NaCl (10). The product was immediately passed through an Ultrogel AcA 34 column (200 ml) (LKB Instruments, Rockville, Md.) in PBS and the fraction of the elution peak corresponding to Fab'2 free of Fc by Ouchterlony analysis was collected. Successive immunoadsorptions were then performed with cyanogen bromide-activated Sepharose 4B columns coupled to: (*a*) pooled normal human sera (500 mg in 20 ml gel), (*b*) human Cohn fraction II (300 mg in 15 ml gel), (*c*) pooled polyclonal normal IgM (200 mg in 8 ml gel), and (*d*) monoclonal IgM (from a patient with Waldenström's macroglobulinemia) without RF activity (4 mg in 2 ml gel).

*Solid phase RIA.* Inhibition of binding in our previously described solid phase RIA for IgM-RF (11) was used to test the activity and the specificity of the anti-id. Briefly, Fab'2 anti-id, serially diluted with borate-buffered saline, pH 8.0, was incubated for 1 h at room temperature with an equal volume (25 μl) of serum or purified RF. Then the anti-id–RF mixtures (50 μl) were added to plastic microtiter wells (Cooke Laboratories, Alexandria, Va.) previously coated with human IgG (60 μg/ml). After overnight incubation at 4°C and washing with borate-buffered saline, binding of IgM-RF was measured by adding $5 \times 10^4$ cpm $^{125}$I affinity-purified goat-Fab'2 anti-human IgM (50 μl) in 1% bovine serum albumin –borate-buffered saline, iodinated by the lactoperoxidase method as previously described (11). In comparative studies of idiotypic specificity, all RF samples were tested at previously determined dilutions that yielded identical binding activities in the RIA. The effect of different dilutions of anti-id on IgM-RF binding was expressed as a percentage of inhibition of the initial RF activity, i.e.,

$$\% \text{ Inhibition} = 100 - \frac{\text{binding activity with anti-id}}{\text{control}} \times 100.$$

The RF control was a mixture of the same RF and normal rabbit Fab'2 IgG. Each assay was done in duplicate. RF binding activity was expressed in nanogram equivalent Sie/ml using a standard curve made with a purified monoclonal RF (Sie) (12).

*HLA typing.* Typing for HLA A, B, and C antigens (using 13 antisera for A, 17 for B, and 4 for C) was performed by Dr. S. Ferrone of the Scripps Clinic with the standard National Institutes of Health microcytotoxicity technique (13).

## RESULTS

*Family tree.* Fig. 1 shows the family tree, the respective latex titers, and HLA antigens detected in available family members.

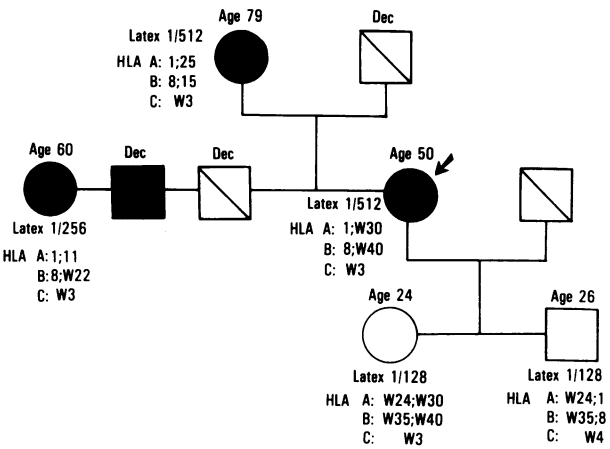


FIGURE 1 Family tree. The patient and the five first degree relatives are shown with the latex titer and the results of HLA A, B, and C typing. The closed circles (females) and squares (males) indicate subjects with definite RA. Dec, deceased; ⊠, ⊘, untested. The arrow indicates the proband.

*Activity and specificity of the anti-id antiserum.* As tested by solid phase RIA, increasing concentrations of anti-id antiserum progressively inhibited the binding of both purified and unpurified Vi IgM-RF up to 90% Table I). Even when added in large excess (10-fold difference), the patient's IgM without RF activity, previously tested by RIA, did not reduce the inhibitory effect of the antiserum on purified IgM-RF binding.

TABLE I
*Activity and Specificity of the Anti-id*

| IgM-RF tested | Dilutions of anti-id 1/64 | 1/16 | 1/4 | 1/1 |
|---|---|---|---|---|
| Polyclonal | | | | |
| Vi | 29±5 | 42±6 | 75±3 | 90±3 |
| Ha | 0 | 0 | 2 | 0 |
| McI | 0 | 3 | 0 | 0 |
| Ph | 0 | 0 | 0 | 0 |
| Me | 0 | 0 | 0 | 0 |
| Jo | 0 | 4 | 2 | 0 |
| Monoclonal | | | | |
| Po | 0 | 0 | 0 | 0 |
| Wo | 0 | 8 | 14 | 30 |
| Ko | 0 | 0 | 4 | 3 |
| La | 0 | 0 | 0 | 2 |
| Sie | 0 | 0 | 5 | 2 |
| Sa | 0 | 0 | 0 | 0 |

The percent inhibition of RF binding was determined by solid phase RIA, as described in Methods. All the RF's were tested with a similar initial activity between 200 and 350 ng equivalent Sie/ml. The results with Vi IgM-RF were calculated from three sets of duplicate RIA, and are expressed with ±1 SD.

To evaluate the specificity of the absorbed antiserum, six different RA sera (Vi, Me, Ph, McI, Jo, Ha) and six purified monoclonal RF (Po, La, Sie, Ko, Sa, Wo) were tested by RIA against the anti-id. All these RF's had an initial binding activity between 200 and 350 ng equivalent Sie/ml. Only one monoclonal RF (Wo) reacted slightly with the undiluted anti-id, but 60-fold less than Vi IgM-RF. Purified RF from four of the same RA sera also did not react with the anti-id.

*Inhibition of first degree relatives' RF binding by anti-id antiserum.* As shown in Fig. 2, increasing amounts of Fab'2 anti-id against Vi IgM-RF progressively inhibited RF binding in the sera of all her first degree relatives. These RF had an initial binding activity between 150 and 220 ng equivalent Sie/ml. The lowest dilution of anti-id assayed inhibited their binding from 40–64%. Identical inhibition patterns were obtained with repeat analysis of the individual sera. The likelihood that all four family members would by chance alone exhibit the same idiotype in their polyclonal IgM RF while none of five successive non-family RA did so, is remote ($\chi^2 = 5.4$, $P < 0.02$).

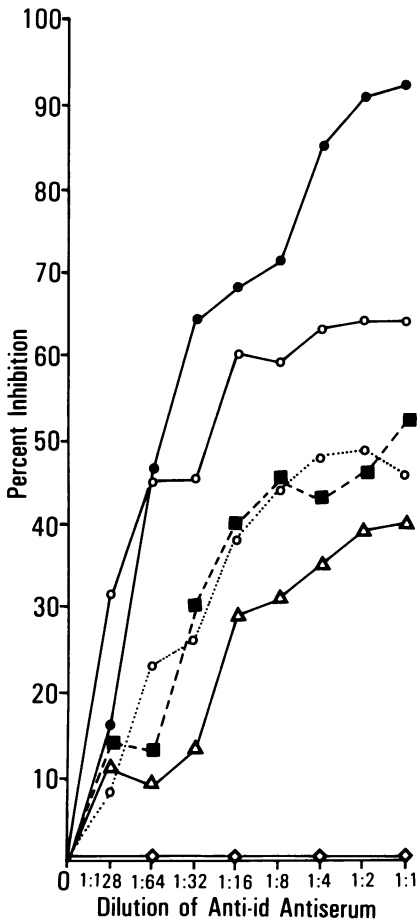


FIGURE 2 Inhibition of RF binding by anti-id against Vi IgM-RF, in four first degree relatives. The percent inhibition is given for each anti-id dilution, as an average result of duplicate RIA. ●, Vi (proband); ■, Mo; ○, Si; ○, Da; △, So; ◇, control (average of five polyclonal RF tested).

## DISCUSSION

Rabbit Fab'2 anti-id antibody against purified IgM-RF from a 50-yr-old woman with classical RA inhibited the RF activity in the sera of her mother (Mo), sister (Si), son (So), and daughter (Da), by 40–64%. The anti-id did not react with the patient's non-RF immunoglobulins, nor with RF from five unrelated subjects with RA. There was no apparent relation between idiotype and HLA.

The IgM-RF idiotypes in the Vi family, detected with our antiserum, are different from the Wa and Po cross-reactive idiotypic determinants described by Kunkel and co-workers (7) on monoclonal IgM anti-γ-globulins from unrelated individuals. Following published procedures, we have also been able to prepare antisera which recognize these cross-reactive idiotypes (14). For the experiments reported here, however, the rabbit antiserum was extensively adsorbed to render it individually specific. The resulting anti-id reacted weakly with only one (Wo) of six monoclonal anti-γ-globulins, including members of both major cross-reactive idiotypic groups.

Idiotypic antigens are serologic markers which only indirectly identify specific portions of the variable regions of immunoglobulin light and heavy chains. Each polypeptide chain is probably coded for by multiple genes that recombine during development (e.g., 15, 16). Thus, the exact interpretation of partial idiotypic identity among family members is rendered difficult in the absence of primary sequence data. It seems unlikely, however, that similar IgM-RF antibody variable regions, as detected by the anti-id antiserum, would be generated exclusively by somatic diversification in the five family members. The results rather suggest the inheritance of antibody genes related to the idiotypic determinants, even though a study of a single family cannot allow an unequivocal conclusion.

From a clinical standpoint, the clustering of five RF-positive individuals in a single family is unusual, and may not be representative of most patients with RA. Methods are now available, however, for the in vitro induction of RF synthesis by lymphocytes from seronegative normal adults (17). Idiotypic analysis of such in vitro secreted RF in other families with RA could reveal whether or not the inheritance of RF idiotypes is a broader phenomenon relevant to the pathogenesis of the disease.

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