

Human Rheumatoid Factor Cross-idiotypes. IV. Studies on WA XId-Positive IgM Without Rheumatoid Factor Activity Provide Evidence that the WA XId Is Not Unique to Rheumatoid Factors and Is Distinct from the 17.109 and G6 XIds

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Summary

The WA cross-idiotype (XId) is the major XId among human monoclonal rheumatoid factors (mRF) and is almost always associated with the light (L) chain XId, 17.109, and the heavy (H) chain XId, G6. A cell line, 35G6, was cloned that bears the WA XId, but shows no reactivity with immunoglobulin G (IgG) and is negative for the 17.109 and G6 XIds. The 35G6 L chain appears to be derived from the same $V_{\kappa}III-J_{\kappa}I$ genes as most WA mRFs L chains. In contrast to the WA mRFs H chains in which $V_{H}1$ genes are used, the 35G6 IgM expresses a $V_{H}3$ gene. Sequence comparisons with other WA XId-positive mRF suggested several common structural features that may be related to the WA XId and differences that may relate to lack of IgG reactivity. Cells similar to 35G6 have previously been described in pokeweed mitogen-stimulated cell lines of peripheral blood lymphocytes from normal individuals and patients with rheumatoid arthritis and type II mixed cryoglobulinemia. These observations were confirmed, and in addition, it was shown that the majority of WA XId-positive cells in these cultures were negative for the 17.109 and G6 XIds. The presence of the WA XId in the absence of IgG reactivity suggests that the WA XId is more directly associated with an antigen specificity other than IgG, and its association with RF activity may be incidental. It is postulated that these WA XId-positive RF-negative antibodies may serve a physiologic role as natural antibodies to a pervasive pathogen, and that IgG reactivity is a consequence of somatic diversification accompanying proliferation of the WA XId-positive RF-negative cell.

The WA cross-idiotype (XId)¹, the major cross-idiotype among human monoclonal rheumatoid factors (mRF), is a conformational antigenic determinant involving both H and L chains and appears to be located in the antigen binding site (1, 2). The L chain-associated XId identified by the mAb 17.109 and the H chain-associated XId detected by the G6 mAb have been reported to occur in almost all WA XId-positive mRFs (3) and has led to the notion that there is restricted expression of these germline genes with little or no

somatic mutation in the WA mRFs. Earlier, it had been postulated that the $V_{\kappa}IIIb$ L chain detected by 17.109 mAb and encoded by the *Humkv325* gene was the structural basis for the mRF WA XId and was unique to IgM with RF activity (4); however, this hypothesis was disproved (2), and it has been shown that the 17.109 and G6 XIds occur separately (5) and together (6) in IgM without RF activity.

Although the WA XId occurs in high frequency among mRF in serum cryoglobulins from patients with type II cryoglobulinemia, it appears to occur in only small amounts among polyclonal RFs in patients with rheumatoid arthritis (7, 8). Although the WA XId has not been reported among monoclonal IgM immunoglobulins without RF activity, among polyclonal RFs, WA XId-positive RF-negative Igs have been reported. WA XId-positive RF-negative plasma cells were detected in PWM-stimulated PBL cultures from normal individuals and patients with rheumatoid arthritis

¹ Abbreviations used in this paper: mRF, monoclonal RF; XId, WA cross-idiotype.

The sequences reported in this paper have been deposited in the GenBank data base under accession numbers M97268 and M87269.

(9) and were also found in synovia of patients with rheumatoid arthritis (10). Moreover, a high incidence of WA XIId antigen has been found in RF-negative sera of patients with juvenile rheumatoid arthritis (11).

In the process of cloning RF-producing cell lines from normal PBL, we cloned a hybridoma cell that produces a WA XIId-positive IgM that does not exhibit RF activity and is negative for the 17.109 and G6 XIDs. The cell line, 35G6, and its secreted IgM are characterized in this study. To determine if the WA XIId-positive RF-negative cells previously observed in normal individuals and patients with rheumatoid arthritis were similar to the 35G6 cells, the original PWM-PBL cultures (9) were reexamined.

Materials and Methods

Cloning of 35G6 Hybridoma, IgM Isolation, and Amino Acid Sequencing of 35G6 IgM. An enriched B cell population obtained from a RF-negative normal donor was transformed with EBV by routine methods. The bulk culture continuously produced a WA XIId-positive RF-negative IgM from day 7 to day 55 and was fused with the F₃B₆ cell line (American Type Culture Collection, Rockville, MD) on day 55. The fusion product was subcloned three times by limiting dilution. The final cell line obtained was monoclonal by gene arrangement and DNA indexing analyses. The 35G6 IgM was isolated from the cell culture supernatant by euglobulin precipitation followed by column chromatography on Superose 6 (Pharmacia, Piscataway, NJ). NH₂-terminal amino acid sequence analysis of 35G6 H and L chains was performed by the Department of Physiology, Tufts University Analytical Core Protein Sequencing Facility (Boston, MA).

Antisera. Goat anti-McD, one of the original antisera to the WA XIId, was raised, absorbed, and characterized (2) in the same way as the rabbit anti-McD (1) (originally anti-Ma). In addition, the F(ab')₂ preparation from the isolated IgG was affinity purified on a WA mRF Sepharose column and further absorbed with solid phase BLA mRF. The final absorbed antisera showed no reaction with non-RF IgM or PO or BLA mRFs by ELISA assay. The antisera has the same specificity as the original rabbit anti-McD and has been the standard polyclonal anti WA XIId typing reagent for the past 15 yr. The monoclonal antisera Glo 108.12 and Glo 86.3 were kindly provided by Dr. D. Posnett (Cornell University Medical School, New York), G6 and G8 that detect V_H1-associated XIId (12, 13) and B6 and D12 that detect V_H3-associated XIId (14) were kindly provided by Dr. R. Jefferis (University of Birmingham Medical School, Birmingham, UK), and 17.109 was kindly provided by Dr. W. Koopman (University of Alabama, Birmingham, AL). RF-TS1 was kindly provided by Drs. I. Randen, K. Thompson, and J. Natvig (Institute of Immunology and Rheumatology, Oslo, Norway).

Assays. WA cross-idiotyping, IgG binding, and immunodot assays were performed as described (2). The Wa and 35G6 IgM binding inhibition ELISA assays were performed in a routine manner using F(ab')₂ goat anti-McD as the ligand. Biotin-labeled Wa mRF and 35G6 were prepared by standard methodologies. The respective biotin preparations that produced a binding of 1 OD were used for inhibition. Either 25 μl of Wa-biotin or 35G6-biotin at 2 μg/ml was incubated with 25 μl of varying concentrations of inhibitor at room temperature in the coated plate for 2 h. The plates were then processed in routine manner. Percent inhibition was determined as follows: 100/OD × (no inhibitor) [OD (no inhibitor) - OD (inhibitor)]. The amount of inhibition that yields

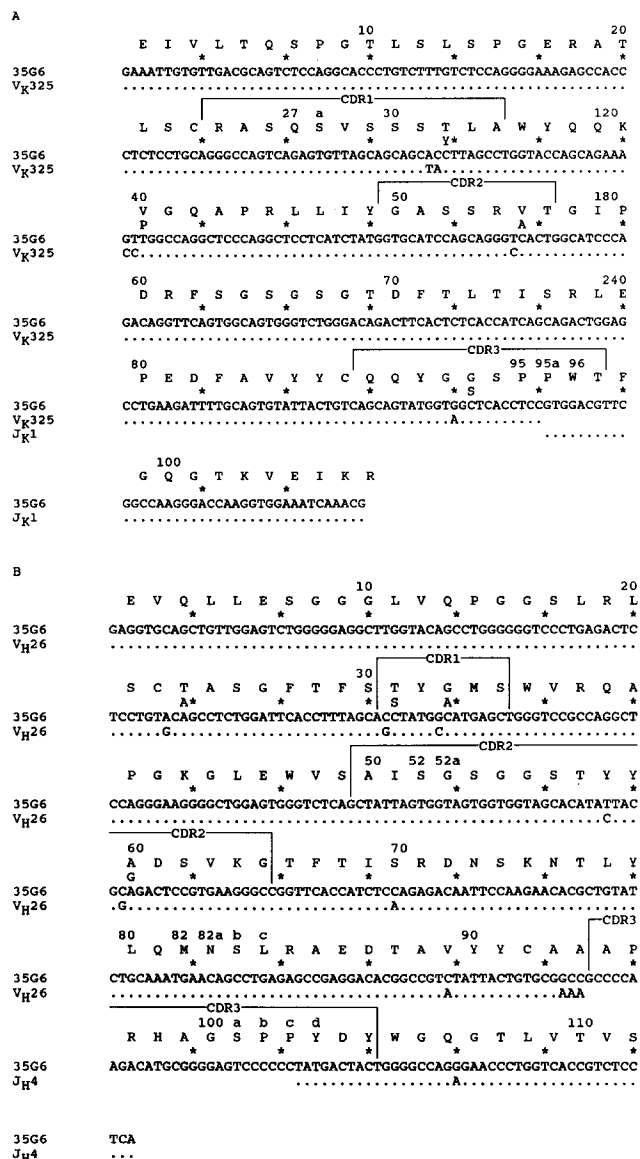


Figure 1. Comparison of 35G6 L (A) and H (B) chain cDNA sequences with their germline counterparts. Both strands of the cDNA were sequenced multiple times. The sequence of the 35G6 cDNA is given. The deduced single letter amino acid sequence is given and numbered according to Kabat et al. (25) above the nucleotide sequence. The sequence of the germline genes is indicated only when a mismatch occurs; homologous regions are indicated by dots. The numbering begins at the initial nucleotide coding for the first amino acid in the mature protein (asterisk every 10 nucleotides). The CDR regions are indicated.

50% inhibition is the I₅₀. Comparison of relative I₅₀ is made by setting the I₅₀ of WA mRF at 1.0.

The competitive inhibition assay for V_KIIIb was performed as previously described (15). The assays for the 17.109 XIId, V_H1-associated XIDs using the G6 or G8 mAbs, and the V_H3-associated XIDs using the B6 or D12 mAbs and Glo antigens using Glo 86.3 mAb were performed in the same way. In addition, Glo 86.3 was tested directly for reactivity with 35G6. The Wa mRF was used as the ligand for the V_H1-XIId assay; the Glo protein was used as the ligand for the 17.109 XIId, V_H3-XIId, and Glo assays. Assays for viral Ab activities (cytomegalic, hepatitis A, B, and C, and HIV-1)

Table 1. Comparison of 35G6 to WA and V_H3 mRFs

mRF	V _H	D _H	J _H	V _κ	J _κ	WA XId
35G6	3*	9‡	4*	3b	1	+
Bor	1	9	4	3b	1	+
Kas	1	9	4	3b	1	+
Sie	1	10	4	3b	1	+
Wol	1	9	4	3b	1	+
RF-TS1	1	11	3	3b	1	+
Lay	3	10	1	1		-
Pom	3	10	1	3a		-
Riv	3	12	6	3a		-

* Subgroup class.

‡ Number of amino acids.

and various autoantibodies (antinuclear, thyroglobulin, microsomal, cytoplasm, DNA, DNA histones, and BSA Abs) were performed by routine methods.

PWM-induced plasma cells and staining of plasma cells for IgG, WA XId, and aggregate IgG binding has been previously described in detail (9). Staining with mAbs 17.109 or G6 were done in the same way except that a fluorescein-labeled F(ab')₂ anti-mouse was used as a second Ab. Slides of cell samples from the original cultures and additional cultures prepared in the same laboratory at about the same time as the originals were stored at -70°C until used in this study.

Oligonucleotides. The following oligonucleotide primers were used: V_H3 FR1, TCCCTGAGACTCTCCTGTGC; V_H3 Leader, GCTGGCTTTTCTTGTGGCT; μ constant (CH1-31), GGA-ATTCTCACAGGAGACGA; μ constant (CH1-24), TCACAGGAGACGAGGGGAA; V_κ3 FR1, GGCACCCTGTCTTTG-TCTCCA; V_κIII Leader, TCCTCTGCTACTCTGGCTCC; κ constant, CTCATCAGATGGCGGAAGAT; and V_H-D junction, TCTATTACTGTGCGGCCGCC.

RNA-PCR and DNA Sequencing. Total RNA was prepared by standard methods. Reverse transcription, using 20 pmol κ- or μ-C region primer or 100 pmol random hexamer primer, and 200 U MoMuLV reverse transcriptase (Superscript RNase H-; Bethesda Research Laboratories, Gaithersburg, MD), and PCR amplification for 30 cycles with a thermal profile of 1 min at 94°C, 1 min at 51°C for H chain primers or 1 min at 55°C for L chain primers, and the V_H-D junction/μ constant (CH1-24) primer pair, and 1 min at 72°C, was performed (16). Asymmetric PCR (17, 18) was performed similarly except that a 1:20 ratio of primers was used for 40 cycles. Both strands of each cDNA were sequenced with Sequenase (U.S. Biochemical, Cleveland, OH) in replicate with each primer. Direct sequencing was employed rather than sequencing cloned PCR products to minimize potential PCR artifacts (18). 35G6 DNA sequences were compared with sequences found in GenBank (release 69).

Results

Characterization of the 35G6 Cell Line and IgM

Protein Analysis. The 35G6 clone produced only IgM_κ immunoglobulin. By NH₂-terminal amino acid analysis, the

first 21 amino acids of the L chain were identical to the product of the V_κIII gene *Humkv325* (19), and the first 19 amino acids of the H chain were identical to the product of the VH3 gene, V_H26 (20).

Sequence of the L Chain. The sequence for the L chain mRNA/cDNA (Fig. 1 A) revealed a close homology with the V_κIIIb germline L chain gene *Humkv325* (19) (97.9% homology). The six point mutations in the coding region resulted in four changes in the amino acid sequence; each of the CDR has a change in amino acid, as does FR2. No mutations were found in the J_κ1 gene. Compared with *Humkv325*, an additional codon was found at the V-J junction.

Sequence of the H Chain. The sequence for the H chain mRNA/cDNA (Fig. 1 B) revealed a close homology with the V_H3 germline gene VH26 (96.6% homology) (20). Ten base changes were observed and three 3' terminal nucleotides of the V gene appear to have been deleted. Four of the changes coded for amino acid changes: one in FR1, two in CDR1, and one in CDR2. The D region could not be assigned to a specific D gene and a J_H4 gene was utilized, which contained two point mutations.

Further confirmation of the sequence for the V-D junction of 35G6 H chain mRNA was obtained by performing an RNA-PCR with an oligonucleotide spanning the V_H-D junction and the μ constant primer (CH1-24) at stringent annealing conditions which would permit amplification only if the V_H-D junction primer annealed along its full length without interruption or mismatch. The anticipated 130-bp product (data not shown) was obtained.

Comparison of 35G6 and WA mRFs V Genes. Previous studies (21-23) of genes associated with the WA XId have noted the essential role of V_κIIIb genes, the preferential use of J_κI, V_H1, and J_H4 genes, and a 9-10 amino acid beginning with glutamic acid and ending with proline. 35G6 similarly utilizes V_κIIIb-J_κI genes and a nine-amino acid D_H ending in proline linked to J_H4 but lacks the initial glutamic acid in D_H and uses a V_H3 instead of a V_H1 gene (Table 1).

The NH₂-terminal J_H4 residue of 35G6 (amino acid 100)

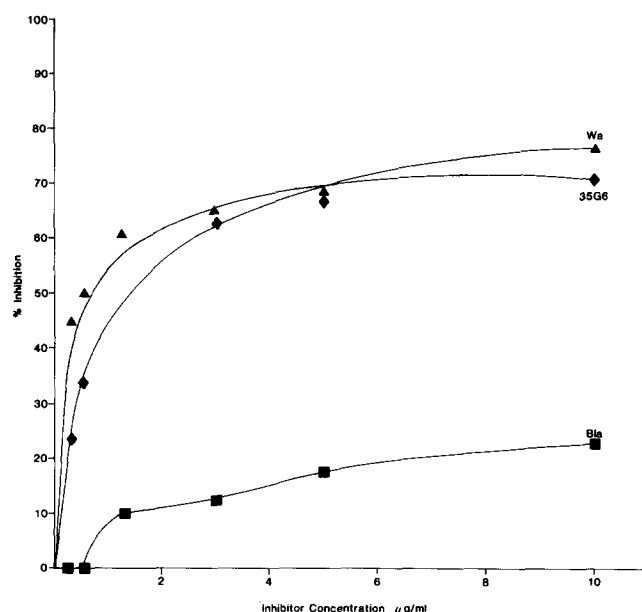


Figure 2. WA cross-idiotype (XId) inhibition assays. Inhibition of polyclonal WA XId reagent, goat anti-McD by varying concentrations of WA mRF (▲), 35G6 IgM (◆), and Bla mRF (■) are plotted.

is tyrosine rather than phenylalanine, but Wol, a WA mRF, also has a tyrosine at this position. The only WA mRF reported to use a J_H gene other than J_H4 is RF-TS1, which uses a J_H3 gene. J_H3 is the same length as J_H4 and bears the closest homology to J_H4 of the J_H genes. The presence of J_H3 does not affect the reactivity of RF-TS1 with G6 or G8 (24) or with the WA XId reagent in this study.

Another difference between 35G6 IgM and other WA proteins is that the arginine (position 94) at the V_H -D junction

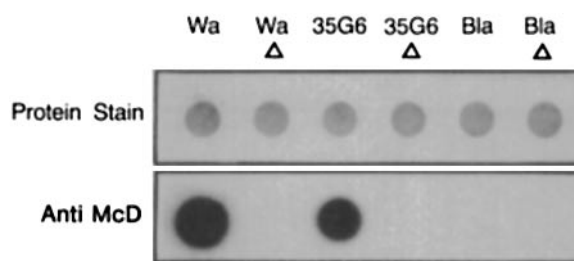


Figure 3. WA cross-idiotype (XId) immunoblot assay. Duplicate nitrocellulose strips were dotted with WA mRF, WA mRF heated (Δ), 35G6, 35G6 heated (Δ), Bla mRF, and Bla mRF heated (Δ). Top strip was stained for protein with Coomassie blue. Bottom strip was probed with goat anti-McD.

has been deleted (Fig. 1 B; GenBank, release 69). The arginine at 94 is also common in PO mRFs (25).

Serologic Studies of the 35G6 IgM. The 35G6 IgM was shown to have the WA XId antigen by both ELISA (Fig. 2) and immunoblot analysis (Fig. 3). Quantitative WA XId analysis showed 1 μ g/ml of 35G6 IgM to give the same inhibition as 0.85 μ g/ml of WA mRF (Table 2). Considering the 10% variation of the assay, essentially all of the 35G6 IgM bears the WA XId. Direct comparison of the presence of WA XId antigen using either Wa mRF or 35G6 IgM binding to the same anti-WA XId reagent (goat anti-McD) showed essentially the same relative I_{50} (Table 2).

35G6 IgM showed no RF activity in concentrations ≤ 100 μ g/ml in an IgG binding assay that could detect as little as 0.01 μ g/ml of mRF and also lacked the L and H chain XId associated with WA mRFs. The 35G6 IgM had $\sim 10\%$ of the reactivity of WA mRFs with the 17.109 mAb by inhibition assay. The weak reactivity of 17.109 with 35G6 IgM was confirmed by the failure to demonstrate reactivity by di-

Table 2. Comparison of Inhibition of WA mRF, 35G6 IgM Binding to Anti-WA XId ($F[ab]_2$ goat anti-McD), Monoclonal 17.109 to Glo mRF, and Monoclonal G6 Binding to WA mRF by Various Monoclonal RFs

mRF Inhibitor	Relative inhibition			
	WA mRF	35G6 IgM	17.109	G6
35G6	1.2*	0.8	9.2	>200
Wa [‡]	1.0	1.0	1.0	1.0
Glo [‡]	0.5	0.5	0.4	>200
Gol	0.6	ND	4.6	135
McD [‡]	0.6	0.6	2.5	4.6
Bla [§]	>10.0	>10.0	>16	>200
Puf	>10.0	>10.0	>16	>200
Sha	>10.0	>10.0	>16	>200

* I_{50} , WA mRF = 1.0.

[‡] WA mRF.

[§] BLA mRF.

^{||} Non-RF mIgM.

rect binding ELISA or immunoblot assays. Glo, Gol, and 35G6 L chain sequences differ from that of Sie (the immunogen for 17.109) by 4, 8, and 11 residues (GenBank, release 69), respectively. Comparison of reactivities of these proteins with 17.109 and anti-WA XIId (Table 2) indicates that a few amino acid changes in the V_HIII L chain can greatly reduce reactivity with 17.109, although they do not affect reactivity with the anti-WA XIId. For example, Glo and Gol had almost identical reactivities with anti-WA XIId, but Gol had <10% of the reactivity with 17.109 shown by Glo. As expected, both 35G6 IgM and Glo mRF did not inhibit G6 binding because both possess V_H3 H chains, but it is notable that, of five WA mRFs, only two were G6 positive, and one of these (McD) was only weakly positive (Table 2). The 35G6 IgM did not inhibit the B6 and D12 V_H3-associated XIId assays and did not react with mAb Glo 86.3, which has been shown to have reactivity with the WA mRFs and Po mRF, a V_H3 protein (26) (data not shown). No viral or autoantigen reactivity was found with 35G6 IgM. The 35G6 IgM did react with staphylococcal protein A, as do most IgM with V_H3 H chains (27).

Immunofluorescence Studies of PWM-stimulated PBLs from Normal Individuals and Patients with RA. WA XIId-positive RF-negative plasma cell cultures from PWM-stimulated PBL from normal individuals and patients with RA (four HL,

BB, NA, and BB from the original study [9] and seven additional cultures) were reexamined for WA, 17.109, and G6 XIId antigens, and aggregated IgG binding (Table 3). The previous findings that WA XIId-positive cells were negative for aggregated binding were confirmed. In the entire study, only a rare WA XIId-positive cell was found with aggregated IgG binding. The percentage of cells that were positive for 17.109 and G6 was similar but substantially less than the observed percentage of WA XIId-positive cells. Cells from patient DE were also tested with anti-McD and mAb 17.109 absorbed in the same manner and with the same normal human serum (NHS) used in the original study, i.e., 12% NHS was added to each antiserum (9). WA XIId-positive cells decreased from 24 to 18%. There was no change in 4% positive 17.109 cells. The relatively small drop in the percentage of WA XIId-positive cells in this study (6%) compared with the sample in the original study (45%) is most likely attributable to the more complete absorption of the antisera in this study, resulting from the more sensitive assays used for the detection of contaminating Abs, i.e., ELISA versus hemagglutinin.

By double-staining analysis, the majority of WA XIId-positive cells were negative for both 17.109 and G6; all cells positive for 17.109 or G6 were also positive for WA XIId (data not shown). The percentages of cells reactive with WA XIId and either 17.109 or G6 were not altered by the order of

Table 3. Cytoplasmic Igs and WA XIId Detected in RA and Control Cells after 6-d Culture of PBL with PWM

RF Patient	Percent plasma cells of total cells	Percent WA XIId positive cells of total plasma cells	Percent 17.109 positive cells of all plasma cells	Percent G6 positive cells of all plasma cells	Percent cells binding aggregated IgG of all plasma cells
	%				
DU	14.5*	33†	7§	7§	0
HL	12.6	15	6	11	0
BB	10.6	10	4	ND	0
FE	11.2	46	4	8	0
DE	25.0	24	4	1	0
QT	10.0	8	0	3	0
AL	36.0	33	14	13	0
VI	11.8	27	3	10	0
Control					
NA	13.3	4	<1	<1	0
BP	15.0	3.3	1.5	1.0	0
VB	14.2	3.5	1.3	0.98	0

* Data indicate the percentage of cells showing cytoplasmic staining with fluorescent anti-human Igs (see Materials and Methods), as counted among 100 total cells identified by phase contrast.

† Data indicate the percentage of cells showing WA XIId stained with anti McD and fluorescein-conjugated second Ab (see Materials and Methods), as counted among 100 Ig-containing cells identified by staining with rhodamine-conjugated antitotal human Ig Abs.

§ Data indicate the percentage of cells stained with either mouse monoclonal 17.109 or mouse monoclonal G6 and fluorescein-conjugated second Ab (see Materials and Methods), as counted among 100 Ig-containing cells identified by staining with rhodamine-conjugated antitotal human Ig Abs.

|| Data indicates the percentage of cells binding fluorescein-conjugated IgG, as counted among 100 Ig-containing cells identified by staining with rhodamine-conjugated antitotal human Ig Abs.

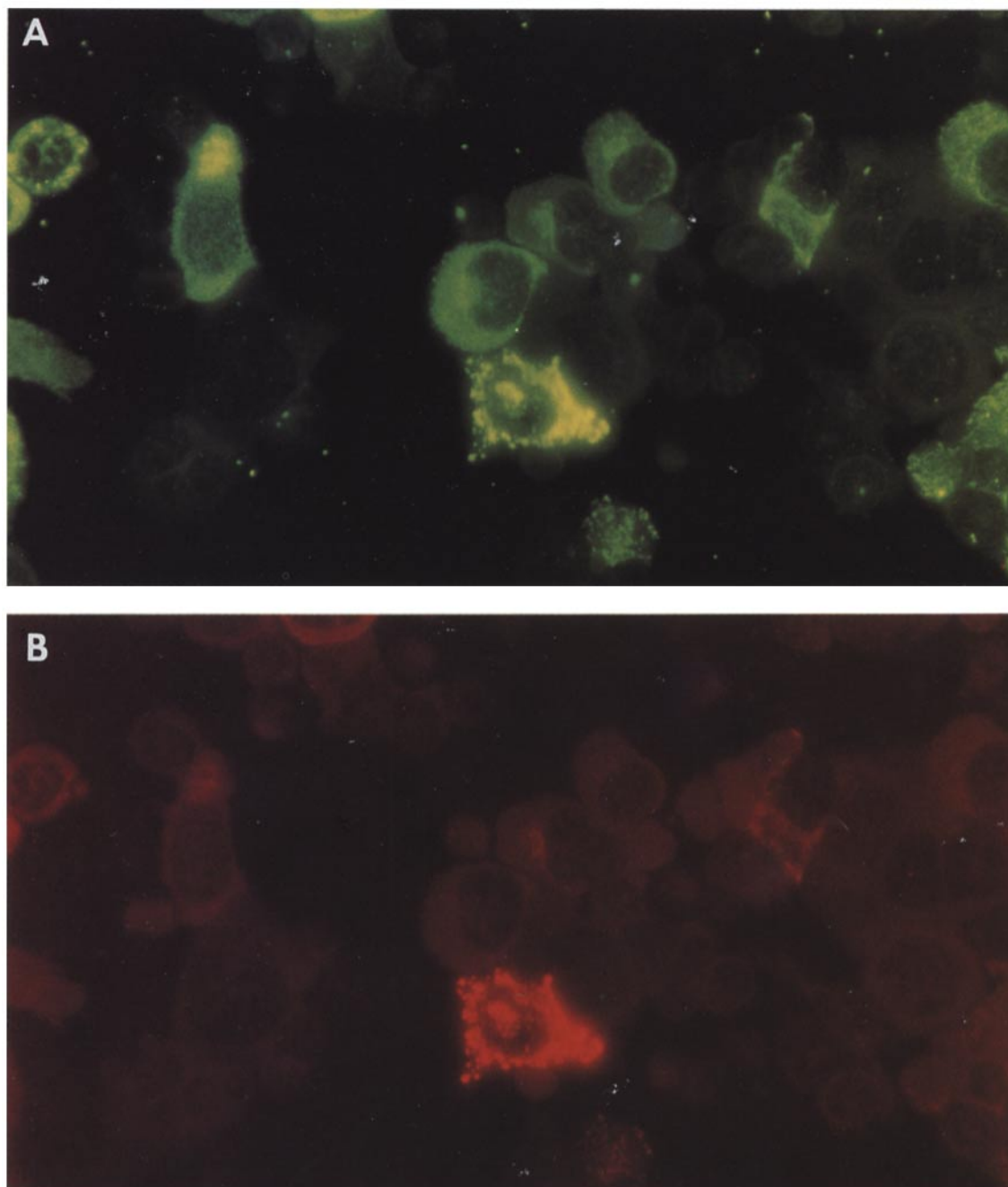


Figure 4. Immunofluorescence of plasma cells from 5 d PWM cultures from PBL from an RA patient. Staining with fluorescein conjugates for WA XIId and rhodamine conjugates for 17.109. (A) PWM cultures, selective illumination for fluorescein; several plasma cells show WA XIId. (B) Same as A: selective illumination for rhodamine; only one cell shows 17.109. $\times 500$.

staining with the anti-McD and mAb 17.109 mAb G6. An example of the paucity of 17.109-positive cells among WA XIId-positive cells is illustrated in Fig. 4.

Discussion

These studies indicate that the conformational H-L chain antigen that constitutes the WA XIId is not restricted to RFs and does not require the presence of the 17.109 or G6 XIDs.

Furthermore, certain V_H3 as well as V_H1 H chains can generate the WA XIId.

The data presented in this study, which demonstrate that relatively few mutations in the *Humkv325* gene markedly decrease the expression of the 17.109 XIId without affecting the WA XIId, indicate that previous conclusions regarding the prevalences of WA mRFs based on serologic studies with mAb 17.109 are not valid. Also, from dual staining immunofluorescent studies with the WA XIId reagent and either 17.109 or

G6 reagent, neither the 17.109 nor G6 epitopes appear to be part of the WA XId epitope(s).

Comparison of 35G6 L and H chain structure with those of WA and other mRFs suggests that the WA XId is determined by structural motifs contributed by a conserved FR3 present on V_H1 and V_H3 H chains, a discrete-sized D region, and use of J_H3 or J_H4 in combination with specific V_HIIIb L chains. Of interest regarding the discrete-sized D region requirement is the recent report that 17.109 positive G6 positive IgM without RF activity produced in transfectomas have marked variability in the length of the D region (6). Because all 17.109, G6 mRFs previously studied have been WA XId-positive, the requirement of the discrete-sized D region can be tested by assaying these transfectoma IgMs for WA XId.

From comparative studies of 35G6 with Wa mRF, IgG reactivity appears to be related to the presence of arginine at position 94 and glutamine at position 95 in Wa mRF H chains. The presence of an extra proline at the V_κ-J_κ junction in 35G6 is against the hypothesis that such an additional proline is important for RF activity (27). An additional amino acid at the V_κ-J_κ junction is relatively uncommon, and it has been proposed that the mechanism for generation of such additional amino acids may be an abnormal process (28). The additional amino acid at this position in a human Ab from normal individuals (29) and in the 35G6 IgM that was generated from a normal individual indicates that the mechanism involved is more likely a normal one.

The original explanation (9) for the observation that WA XId-positive RF-negative cells are present in PWM-stimulated (PBL) cultures was that pentameric IgM capable of binding aggregated IgG was not present in these cells. Our finding that the 35G6 cell line produces a WA XId-positive RF-negative IgM suggests an alternative explanation that the WA XId-positive RF-negative cells are progenitors of the cells that do produce WA XId-positive RF-positive IgM. The high prevalence of WA XId-positive cells in PWM-PBL cultures compared with the prevalences of 17.109-positive and G6-positive cells may be due to somatic mutation of the germline genes that encode the 17.109 and G6 XIds or may indicate that other genes encode the WA XId. The data demonstrating that relatively few mutations in the *Humkv325* gene greatly decrease the expression of 17.109 XId without affecting the WA XId, and the rarity of expression of genes other than *Humkv325* in Wa mRF, favor the former possibility.

It was initially thought that increased prevalence of WA XId-positive plasma cells in patients with rheumatoid arthritis may be related to the disease process, but small amounts of 17.109 XId and G6 XId found in the blood were interpreted as against this possibility (4). The finding that with somatic

mutation the 17.109 XId is lost and the WA XId retained, coupled with studies that demonstrated overexpression of somatically mutated *Humkv325* genes in synovial tissue (28), indicate that the absence of 17.109 and G6 XIds does not exclude Wa mRF.

Pernis et al. (10) have suggested that the WA XId-positive RF-negative cell represents a regulatory cell type. The presence of a molecule with the WA XId but without IgG reactivity would permit modulation of anti-WA XId without the presence of RF in the circulation. The identification of WA XId-positive RF-negative cells in normal individuals and in patients with rheumatoid arthritis may be consistent with the hypothesis that the WA XId-positive RF-negative IgM cell modulates suppression of RF production. The detection of WA XId-positive RF-negative IgM cells in patients with juvenile rheumatoid arthritis may reflect this suppression (11). However, if this hypothesis were correct, the WA XId-positive RF-negative cell would be absent or reduced in patients with essential mixed cryoglobulinemia; this is not the case (9).

An alternative hypothesis is that the WA XId-positive RF-negative cell has a physiologic role, which is the secretion of natural Ab to a common pathogen, and that RF activity is a cross-reactivity that develops with somatic mutation accompanying proliferation of these cells. Both the serologic data on Wa mRFs and the immunofluorescent studies on the PWM-stimulated cells indicate that the WA XIds are preserved in the presence of mutation of the germline genes that encode the 17.109 and G6 XIds. If the main association of cross-idiotypes is with antigen specificity of the Ab as first described (30, 31), then conservation of the WA XId suggests an antigen selection process for WA XId-positive cells. The recent finding of chronic hepatitis C virus (HCV) infection in patients with mixed cryoglobulinemia (32) suggests that HCV may be the pathogen that stimulates proliferation of the WA XId-positive RF-negative cells.

The mRFs encoded by germline genes have been found to cross-react with non-IgG antigens (15, 33). The finding that random pairing of 17.109 L chains and G6 H chain did not produce IgM with RF activity and that certain somatic mutations of CDR3 are required for generation of reactivity with IgG and other self-antigens (6) is consistent with the possibility that the germline genes that encode the 17.109-positive G6-positive IgM are preserved by selection for reactivity with antigens other than IgG. Hence, the RF activity of WA mRFs may be incidental and unrelated to the physiologic role of these Abs but may play a role in disease as a result of untoward proliferation and involvement in immune complex-mediated inflammation.

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