Brief Definitive Report

HUMAN RHEUMATOID FACTOR CROSSIDIOTYPES

II. Primary Structure–dependent Crossreactive Idiotype, PSL2-CRI, Present on Wa Monoclonal Rheumatoid Factors Is Present on Bla and Other IgMκ Monoclonal Autoantibodies

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The major human monoclonal rheumatoid factor (mRF) crossidiotype (XId) Wa is present on ~60% of mRFs (1). Distinct from Wa are the two minor groups, Po and Bla (1, 2). The three XIds were initially defined serologically using polyclonal antisera raised to native mRFs. Subsequently, by using antisera raised to synthetic hypervariable region (complementarity determining region [CDR]) peptides of a Wa group mRF (Sie), a primary structure–dependent XId was defined that was thought to be unique to the Wa group (3–5). Analysis of light chain amino acid sequences of Wa mRFs revealed that only the second CDR (L-CDR2) was identical in all groups. The crossreactive idiotype (PSL2-CRI) was defined using an antiserum to the second CDR peptide of the Sie light chain (anti-PSL2). Anti-PSL2 reacted with all Wa mRFs tested and only weakly and not at all, respectively, with the proteins Pom and Lay of the Po group. These and other data obtained were consistent with the possibility that the PSL2-CRI was unique to the Wa mRFs. However, in a study of amino acid sequences of κIIIb light chains of monoclonal human IgMκ autoantibodies, an antibody with specificity for low-density lipoprotein (Son) had the same L-CDR2 amino acid sequence as the Wa mRFs (6). This protein did not have anti-γ-globulin activity and did not bear the Wa XId (7). These data suggested that the PSL2-CRI may not be confined to Wa mRFs. The third mRF XId group (Bla) was not investigated in the initial PSL2-CRI studies. Recent study (8) has demonstrated the presence of PSL2-CRI on Bla mRF, the prototype of the Bla group. The present study was undertaken to determine the amino acid sequence of the L-CDR2 of Bla, to compare it with the L-CDR2 sequence of Wa and Po mRFs, and to determine whether the PSL2-CRI is present on other monoclonal autoantibodies. Preliminary reports (9, 10) of these studies have been published.

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Materials and Methods

The isolation and idiotypic characterization of the IgMκ mRFs Wa and McD of the Wa group, Lay of the Po group, and Bla of the Bla group have been described in detail previously (1, 2). Isolation of the IgMκ monoclonal anti–low-density lipoprotein (Son) and analysis of light chain amino acid sequences have also been reported previously (6). Sera of Joh and Gag, IgMκ monoclonal cold agglutinins, were provided by Dr. R. Williams (Univ. of New Mexico Hospital, Albuquerque, NM). Partial analyses of the amino acid sequences of the light chains of these two proteins have been performed previously, and both characterized as VκIII (11). The monoclonal cold agglutinins were isolated from serum by high-pressure liquid chromatography using 5000-PW column 7.5 mm × 30 cm (Altex Spherogel-TSK; Beckman Instruments, Inc., Fullerton, CA). Immunoblots of denatured light chains isolated by SDS-PAGE were tested by probing Western blots of the gels as reported (8). Anti-PSL2 and anti-PSL3 (4) were provided by Drs. P. P. Chen and D. A. Carson (Scripps Clinic, La Jolla, CA). Goat anti–human κ chain serum was obtained from Kallestad Laboratories (Austin, TX). Enzymatic digestion, purification of peptides, and analysis of amino acid sequences were performed as described (12).

Results and Discussion

The amino acid sequence of the L-CDR2 of the Bla protein shown in Fig. 1 was identical to that reported for the Wa mRFs. The reactivity of anti-PSL2, anti-PSL3, and anti-κ with denatured light chain of the various monoclonal autoantibodies is shown in Fig. 2. Anti-PSL2 reacted with all light chains except those from Lay, the Po mRF. Anti-PSL3 reacted with light chains of the Wa mRFs, Wa and McD, and Son, the anti–low-density lipoprotein, but not with the light chains of Bla or the cold agglutinins.

The demonstration in this study that the L-CDR2 of Bla mRF is identical to that of the Wa mRFs and that the PSL2-CRI, as determined by anti-PSL2, is present on Bla, a mRF that reacts with DNA nucleoprotein, Son, a monoclonal anti–low-density lipoprotein, and two monoclonal cold agglutinins unequivocally rules out that the PSL2-CRI is associated only with mRFs of the Wa group. On the contrary, these findings suggest that the PSL2-CRI may be a common determinant on most IgMκ monoclonal autoantibodies and raise the possibility that the PSL2-CRI is an idiotype involved in a more general regulation of autoantibodies. The high incidence of PSL2-CRI among rheumatoid factors in essential mixed cryoglobulinemia, a disorder in which the rheumatoid factors are usually monoclonal, also supports the hypothesis that PSL2-CRI is associated with monoclonal IgMκ autoantibodies (4).

A major alternative possibility to that of selective association of PSL2-CRI with monoclonal autoantibodies is that the presence of PSL2-CRI among monoclonal autoantibodies reflects the occurrence of the PSL2-CRI in the IgMκ pool in pathologic sera. This must be considered for several reasons. A high incidence of PSL2-CRI occurs among VκIIIb light chains (4, 6); VκIIIb light chains make up a substantial portion of κ light chains of polyclonal IgM in normal serum, whereas only trace amounts are present among light chains of IgG and IgA (13). Uncertainty exists about the percentage of VκIIIb light chains present among IgMκ proteins (13, 14) and about the effect of polyclonal increase of IgM on VκIIIb concentrations (15). Therefore, the high incidence of PSL2-CRI among monoclonal autoantibodies may reflect the relatively high levels of IgMκIIIb that may be present in pathologic sera.

	CDR2						
	Amino Acid Position						
	50	51	52	53	54	55	56
Bla	Gly	Ala	Ser	Ser	Arg	Ala	Thr
Wa group							
Wol	—	—	—	—	—	—	—
Sie	—	—	—	—	—	—	—
Gar	—	—	—	—	—	—	—
Got	—	—	—	—	—	—	—
Neu	—	—	—	—	—	—	—
Flo	—	—	—	—	—	—	—
Po group							
Lay	—	—	—	Thr	—	Glu	Ala

FIGURE 1. Bla light chain CDR2 amino acid sequence compared with reported Wa and Po mRF CDR2 sequences (12).

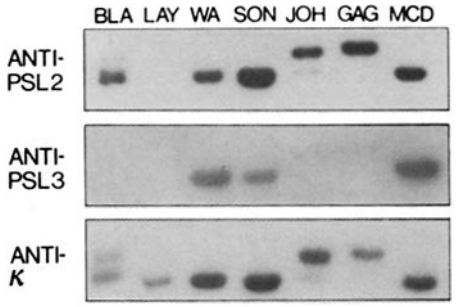

FIGURE 2. Triplicate immunoblots of denatured light chains from Bla mRF, Lay mRF, Wa mRF, Son anti–low-density lipoprotein monoclonal antibody, Joh monoclonal cold agglutinin, Gag monoclonal cold agglutinin, and McD mRF. Blots are probed with anti-PSL2, anti-PSL3, or anti-κ, then with ^{125}I-labeled protein A.

Whether VκIIIb and the PSL2-CRI are selectively present in autoantibodies could possibly be determined more definitively from studies of polyclonal autoimmune disease states. Thus far, studies on polyclonal rheumatoid factors are conflicting. Qualitative studies have shown a high incidence of PSL2-CRI, whereas quantitative studies for VκIIIb indicate that only small amounts are present (16, 17). From the evidence presented here and previous studies, which demonstrated that the Wa and Bla XIds are conformational antigens requiring both heavy and light chains, it is clear that the determinant detected by anti-PSL2 is primary structure antigen not directly related to the Wa or Bla XId determinants. It remains to be determined whether PSL2-CRI is indeed an XId that is selectively present on autoantibodies or an allotypic marker for a VκIII germline gene. The latter has not been excluded because somatic mutation of the L-CDR2 could explain the presence of VκIII light chains that do not react with anti-PSL2.

Summary

The amino acid sequence of the L-CDR2 (complementarity-determining region) of Bla mRF (monoclonal rheumatoid factor) is identical to that of the Wa mRFs. The PSL2-CRI (crossreactive idiotype), as determined by anti-PSL2, which has been shown to be present on all Wa mRFs, is also present on the Bla mRF and other monoclonal autoantibodies. PSL2-CRI is, therefore, not unique to Wa mRFs and may be present on most IgMκ monoclonal autoantibodies. Whether PSL2-CRI is a crossidiotype (XId) that is selectively present on autoantibodies or represents an allotypic marker for a VκIII gene is undetermined.

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