To the editor:

WA monoclonal rheumatoid factors and non-Hodgkin lymphoma

De Re et al describe the sequences obtained from B-cell monoclonal expansion analysis of patients with non-Hodgkin lymphoma (NHL).1 The characterization by the authors of 17 patients with NHL makes comparisons of the antibodies found to rheumatoid factor genes and hepatitis C virus (HCV) anti-E2 antibodies. The study did not include serologic or sequence determinations demonstrating identity between the serum monoclonal rheumatoid factors (mRFs) and the immunoglobulins (Igs) antigen receptors of the lymphoma cells in the patients. Sequence data can be useful in attempting to understand the origin of NHL in patients with chronic HCV infection, but there are pitfalls in drawing conclusions on specificities of antibodies based on sequence homologies to only
part of the sequences that constitute the combining site. The sequence homology analyses in this study were flawed. Consequently, the conclusions that malignant cells in type II cryoglobulinemia are derived from RF-producing cells may be only partially correct, and the speculation that HCV E2 protein is involved in the production of RF antibody is not warranted by the data presented.

Because there are extensive sequence and other criteria for mRF bearing the WA cross-idiotype (WA mRF), the predominant mRF in type II cryoglobulinemia,\(^3\) in the absence of demonstrating identity between the serum mRF and the malignant cell Ig antigen receptor, these criteria may be used to provide indirect evidence that the malignant cells arose from the major mRF-producing cells that proliferate during the nonneoplastic course of type II cryoglobulinemia. WA mRF bear a combining-site cross-idiotype (WA) that requires both light- and heavy-chain V-region sequences. Typically, the WA cross-idiotype arises from coexpression of a VH1 gene (DP-10), a D region (D21/9) that encodes 9-12 amino acids beginning with glutamic acid and ending in a proline, and JH4 with a V\(_\text{S3} \) (Kv325) rearranged to J\(_\text{g1} \). Although VH1 is most common, VH3 can be used (eg, M7-RF-WA; GenBank accession number U03400); the latter group differs from the classic WA in that a VH3 gene (DP-54) rearranged to J\(_\text{g3} \) or J\(_\text{g4} \) is coexpressed with Kv328 rearranged to J\(_\text{g1} \). The heavy chain CDR3 amino acid sequence, 13 amino acids long, differed significantly from the classic WA. Thus it appears that there are 2 classes of WA mRF.\(^2,^3\) The speculation that De Re et al made regarding HCV E2 protein is untenable because neither class of WA mRF has any significant homology with the anti-E2 HCV antibodies cited.

Of the 17 immunoglobulin sequences studied by De Re et al, only 4 fit criteria for the WA mRF. The homologies of the sequences deduced in their Table 4 were arbitrary. The E value scores are listed for each IgH CDR3-deduced amino acid sequence to give legitimacy to the assignment, but this was used improperly. The inclusion of the J\(_\text{H} \) sequence shifts the score higher and muddles the interpretation by making the similarities appear more significant than they are. Moreover, as noted above, sequence homology is not the only criterion for WA mRF. The translated D-region sequences of the CDR3 from classic WA often bears little homology. But the structural conformation imposed by the size of the D region, the presence of critical D-region amino acids at precise locations, and the restricted use of specific immunoglobulin genes generate the WA mRF. Thus among the patients 1-4 that were considered by De Re et al to be similar to WOL (a classic WA mRF), only the patient 1 sequence bears similarity to WOL because the others lack critical D-region amino acids. Patients 5, 6, and 10 appear to be similar to M7-RF-WA (accession number U03400). The IgH from patient 13 does not appear to be similar to BOR (a classic WA mRF), based upon the CDR3 sequence and the presence of a VH4 gene instead of VH1, which makes it more similar to the cold agglutinins. The patient 14 IgH was reported to have a CDR3 similar to a mouse rheumatoid factor, RF-MR20.

Establishing lineage of malignant cells to mRFs requires the demonstration of identity of the Ig antigen receptor and the preneoplastic mRFs. The data presented by De Re et al did not accomplish this, but the presence of 4 WA mRF NHLs did provide additional indirect evidence for the original hypothesis that HCV is responsible for proliferation of a specific set of B cells in patients with type II cryoglobulinemia and that transformation to malignant cells occurs in some of these cells.\(^4\)

Although HCV is not an oncogenic virus, HCV nonstructural protein NS3 and HCV core protein have been reported to transform cells in vitro. It remains to be determined if the malignant transformation of the HCV-driven proliferating B cells is a stochastic event, a result of a subset of these B cells prone to malignant-transforming mutational events, or a direct effect of the virus.

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References


**Response:**

**Relationship between IgR expressed by hepatitis C virus–associated non-Hodgkin lymphomas and rheumatoid factors**

The primary aim of our study\(^1\) was to demonstrate that a large proportion of hepatitis C virus (HCV)–associated non-Hodgkin lymphomas (NHLs) derive from B-cell clones chronically stimulated by a common antigen. This is deduced on the basis of peculiar properties that these NHLs express: intrachordal diversity, an R/S mutation ratio in the FR segments of IgR lower than expected by chance, and a highly restricted use of gene segments in assembling IgR. We believe that our data support the proposed pathogenetic model adequately.

Concerning the derivation of the NHLs from B-cell clones that produce rheumatoid factors (RFs), this is only supposed in our study (the title reads “Sequence analysis of the immunoglobulin antigen receptor of hepatitis C virus–associated non-Hodgkin lymphomas suggests...”) on the basis of the significant homologies found between gene segments (both V\(_\text{H} \) and V\(_\text{K} \)) used by the NHLs in assembling IgR and gene segments used by antibodies with RF activity. It is worth noting that 6 of 9 patients with a previous history of open mixed cryoglobulinemia (MC) syndrome, but none of the 8 patients without MC, specifically used the D21/9 segment gene, a region also frequently used by the WA cross-idiotype of RF found in MC. This finding supports a possible correlation between NHLs and B-cell clones producing RFs. Moreover, we previously demonstrated that premalignant and malignant lymphoproliferations in an HCV-infected type II MC patient were sequential phases
of a unique antigen-driven pathologic process.\textsuperscript{2} This finding again supports the possibility that the NHL originated from a B-cell clone that was already present and antigenically stimulated at the time of MC onset.

We agree that a formal demonstration of the identity of HCV-associated NHLs with B-cell clone(s) that produce RFs could be derived only by a direct comparison of the amino acid (AA)–deduced sequence of the IgR expressed by the lymphomatous clone with the AA sequence of the specific RFs present in the sera of each single patient. But this was beyond the scope of our study. Nevertheless, it is our intent to prove this point by comparing the AA-deduced Ig sequence obtained from serum of some of these patients with their NHL IgR sequence.

Concerning the suggested implication of HCV as the pathogenic agent of the HCV-associated NHLs, this was derived from the analysis of sequence homologies between the IgR expressed by the NHLs and that of antibodies specific for the E2 protein of HCV. In our opinion, such a hypothesis is interesting in the light of the almost complete association and supposed pathogenetic relationship between HCV infection and MC syndrome, which precedes NHL onset, at least in a large group of NHL patients. Although such a hypothesis needs a formal demonstration, it is worth noting that Chan et al\textsuperscript{3} have recently reported that HCV-associated NHLs and normal B cells responding to E2 viral antigen preferentially use the VHI-69 gene, which is also used by some NHLs we analyzed (VHI-69 is synonymous with VH1/DP1O, and VHI/DP-1O and VHI/DP-88 genes differ in only 1 nucleotide) and is typically used by RF-WAs present in MC. Thus these data indicate that some RF-WAs may have an anti-HCV specificity. Moreover, in HCV infection the reactivity of IgM with the corresponding IgG is inhibited by the addition of HCV antigens, suggesting that the antigen-binding site of the IgM is cross-reactive with HCV antigens.\textsuperscript{4} Furthermore, IgG-IgM WA immune complexes were found in HCV-infected patients but not in acute and chronic hepatitis B and acute hepatitis A infections. Thus IgG-IgM WA immune complexes appear to be uniquely associated with HCV infection, supporting the possibility that they derive from an antigen-driven response closely related to the virus.\textsuperscript{5}

Finally, since antibody specificity is primarily dependent on the CDR3 region, which is the most variable part of the V region, we have limited the search for sequence homologies to this part of the IgR region.\textsuperscript{1} But the results of database research using the entire AA-deduced V sequence again confirms significant homologies with some RFs in most of the cases. Concerning the IgR sequence reported for patient 13, we agree that it may not be similar to that of RF-Bor (the E value was high). In contrast, the homology reported for patient 14 with RF is valid, RF-MR20 being a human rheumatoid factor.

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References


To the editor:

Clarifications to the standard neutrophil response criteria for clinical trials in myelodysplastic syndromes are needed

The recent article by Cheson et al\textsuperscript{1} represents an important step toward standardizing the response criteria used in clinical trials of new therapeutic agents for patients with myelodysplastic syndromes (MDSs). We believe that 3 clarifications are necessary to the proposed neutrophil response criteria to avoid classifying patients with spurious increases in neutrophil count as having responded to therapy and to appropriately classify patients who enjoy a genuine, physiologically relevant increase in neutrophil count.

The international working group’s proposed criteria for the increment in peripheral blood counts necessary to qualify as a minor erythroid response and minor platelet response incorporate minimum absolute increments of 1 g/dL and 10 000/mm\textsuperscript{3} (for transfusion-independent patients), respectively. The proposed minor neutrophil response criterion, in contrast, does not incorporate a minimum absolute increment but simply requires an increase in the absolute neutrophil count (ANC) of at least 100%, but absolute increase less than 500/mm\textsuperscript{3}.\textsuperscript{1(p3672)}

For patients with very low neutrophil counts, the lack of an absolute minimum increment required in order to meet the minor criterion may be problematic. Many factors contribute to day-to-day variability in the neutrophil count in normal persons, and we have also observed such day-to-day variability in patients with MDS in the absence of any specific intervention directed at the neutrophil cell line. The time of day at which blood is drawn for analysis,\textsuperscript{2} the degree of recent physical exertion,\textsuperscript{3} and (for premenopausal women) the phase of the patient in the menstrual cycle\textsuperscript{4} are just some of the many factors that can contribute to daily variability in ANC. The precision and accuracy of laboratory determinations of the ANC in patients with very low neutrophil counts may also be questionable.\textsuperscript{5} A hypothetical patient with MDS who began a clinical trial with an ANC of 100/mm\textsuperscript{3} and subsequently was found to have an ANC of 200/mm\textsuperscript{3} could be listed as a minor neutrophil response according to the current criterion, but such an improvement may not be meaningful or reproducible.

The proposed major neutrophil response criterion, “For absolute neutrophil count (ANC) less than 1500/mm\textsuperscript{3} before therapy, at least a 100% increase, or an absolute increase of more than 500/mm\textsuperscript{3}, whichever is greater”,\textsuperscript{1(p3672)} is cumbersome. We understand a need for a clearly defined absolute minimum increase in ANC but do not appreciate a need for the additional inclusion of a minimum percentage increase. A hypothetical patient with MDS who began a trial with an ANC of 1000/mm\textsuperscript{3} and whose ANC
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