EDITORIALS

RHEUMATOID FACTORS AND GERMLINE GENES IN RHEUMATOID ARTHRITIS: EVIDENCE OF AN INTRINSIC B-LYMPHOCYTE DEFECT?

ADVANCES in molecular biological and human cell hybridization technology significantly enhanced our knowledge of human immunoglobulin variable region (IgV) gene organization, structure and expression. These advances have also provided insight into autoimmunity and mechanisms that may determine the nature of the antibody repertoire expressed in RA patients.

Since the specificity for antigens is determined by the precise sequence of amino acids used in the antigen-binding site, studies in autoimmune diseases have focused on the combinatorial events and mutations which occur in genes that encode autoantibodies. Explicitly, the question has been whether autoantibodies are direct copies of germline genes, or products of somatic mutations. Non-random distribution of replacement mutations in rearranged IgV genes, compared with their germline counterparts, is indicative of antigen-driven selection of B-lymphocytes [1]. In contrast, the lack of mutations, or evidence of limited random mutations, is indicative of non-specific polyclonal activation. IgV genes have also been used as markers of clonal relatedness of autoantibodies because unique combinations of V<sub>H</sub>, D<sub>H</sub> and J<sub>H</sub> or V<sub>L</sub> and J<sub>L</sub> elements, formed during B-lymphocyte differentiation and maturation are retained by cells derived from the same progenitor pre-B cell [2].

The evidence from examining IgV gene sequences in RA patients shows that almost all IgM RF and 50% of IgG derived from B-lymphocyte hybridomas or EBV-lines producing monoclonal immunoglobulins are encoded by genes in their germline configuration, or with very few nucleotide changes from known germline genes [3-5]. Interestingly, studies of B-lymphocyte ontogeny have revealed that the same genes are also widely transcribed in early foetal and neonatal B-lymphocytes [6,7]. Moreover, these studies suggested that during early life only a restricted set of genes from a large pool of available genes are used, and that the repertoire diversifies progressively after birth until a normalization of IgV gene use is achieved [8]. One explanation for restricted gene use during early life is that these genes are of special survival value and function before more specialized adaptive responses involving somatic diversification permits the generation of antibodies able to recognize the extensive range of environmental antigens. Recent studies of B-lymphocyte repertoire in normal individuals suggested that B-lymphocytes expressing these genes may also participate in interconnections between T- and B-lymphocytes to maintain homeostasis within the immune system [9]. The overexpression of these genes in RA synovial tissue however, may imply that such IgV genes can be involved in autoimmune diseases in adults.

There are a number of explanations that may account for this outcome in RA. A failure of maturation of the primary foetal B-lymphocyte repertoire in RA patients may result in the over-expression of foetal-type B-lymphocytes [10]. Alternatively, it is possible that a subset of B-lymphocytes is induced to proliferate via surface Ig receptors as a result of exposure to a particular antigen or idiotype or because of an intrinsic defect in regulation of pre-B cells.

Many fundamental questions are raised by these observations and await elucidation. For example, it is not clear how representative synovial hybridomas and EBV-lines are of the RF and immunoglobulins produced in vivo. Furthermore, the factors that result in the breakdown of tolerance to autoantigens in these cells is unknown.

The possibility that cell fusion, transformation or cloning of cultured hybridomas may introduce a bias in IgV analysis is an important issue that needs serious consideration. It is known, for example, that EBV infects B-lymphocytes in the G0 to early G1 phase (resting-premitotic cells) of the cell cycle but not in the S-phase (dividing cells) [11]. Furthermore, terminally differentiated plasma cells, which represent a significant proportion of cells of the B-lymphocyte lineage in RA synovia, can neither be transformed nor fused with fusion partners.

The second issue, that has a significant implication for our understanding of disease mechanisms, is lack of knowledge of the pathway through which these cells are induced to break their immunological silence and produce RF. The fact that B-lymphocyte tolerance exists has been firmly established [12]. Recent studies, using transgenic animals, have shown that tolerance is dependent on both deletion and functional inactivation (anergy) of autoreactive B-lymphocytes and that clonal deletion occurs both in immature and mature lymphocytes that have emigrated from the bone marrow [13]. In addition, it has been suggested that B-lymphocytes expressing foetal-type (or natural) RF can efficiently present antigens complexed to antibody but produce no antibodies in transgenic mice [14]. To test the possibility that regulatory elements play a role in the induction of RF production by these cells, the normal transgenic mice were crossed with the autoimmune MLR-lpr mice. The investigators found a dramatic increase (<200 fold) in the level of serum RF. Although the precise cause of this upregulation of RF production is not known, these investigators attributed the increase in RF production to abnormal genes derived from the MRL strain [14].

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To address these unanswered questions, we have recently tried to bypass the need for EBV transformation and hybridoma generation by examining the serum of monozygotic twins with RA for the expression of germline gene encoded molecules. Monozygotic (MZ) twins, from the Arthritis Research Council’s repository in Manchester, were chosen for this study so as to evaluate the contribution of genetic factors in understanding the mechanisms involved in RF production. As probes we used murine monoclonal antibodies with specificity for cross-reactive idiotypes (CRI) present on RF in foetal and synovial B-lymphocytes. Previous structural and molecular characterization had demonstrated that these monoclonal anti-CRI antibodies reacted with epitopes present on immunoglobulins which were encoded by individual, or a small number of highly related, IgV genes. Mindful of the technical problems associated with the detection of different isotypes of Ig and RF, we opted for extensive fractionation of sera to separate IgM, IgG and IgA with RF activity. The level of expression of the different IgV gene products were determined using highly specific ELISA protocols [15].

The data demonstrated that compared with previously examined normal individuals the serum of the RA twins contained relatively high levels of RF-CRI. Furthermore, it appeared that 46% of IgM RF was encoded by a relatively small set of germline, or minimally mutated germline IgV genes from the VH1,3 and 4 families. Furthermore, a significant proportion of IgG RF and IgA RF (~30%) was also encoded by these same genes. The level of expression of individual germline IgV markers was similar within twins but dissimilar between unrelated twins irrespective of disease status. These data suggested that the high level of expression of the IgV markers is a genetic trait not secondary to disease. However, since different genes are observed in association with RF activity in unrelated twins a selective process appears to operate on RF diversification. Furthermore, from these studies it appears that there are qualitatively two different classes of RF. The first, similar to naturally occurring RF, are encoded by unmutated, or minimally mutated, IgV genes but show evidence of isotype switch from IgM to IgG and IgA. The second class of RF, found in RA patients, are usually of the IgG or IgA isotype and may be with evidence of somatic mutation.

It is possible that the production of RF characteristic of RA patients occurs through a two stage mechanism whereby preactivation of the natural RF repertoire precedes the generation of pathogenic RF. In normal individuals, natural RF expressing B-lymphocytes could be regulated by mechanisms that involve complex interactions with antigens and T-lymphocytes. These B-lymphocytes, which only transiently deviate from a resting state, can capture and present complex autoantigen to autoreactive T-lymphocytes [14]. Recent studies, from a number of laboratories, suggest that when resting B-lymphocytes present antigens to CD4 T-lymphocytes, tolerance results [16]. This has been attributed to the lack of co-stimulatory signals on resting B-lymphocytes. In RA on the other hand, RF expressing B-lymphocytes would be in a state of chronic activation and can provide co-stimulatory signals to autoreactive T-lymphocytes, thus leading to their activation. This B-lymphocyte subpopulation may also lead to the diversification of the autoimmune response because of the larger range of peptides that can be presented by these cells to T-lymphocytes, compared with other antigen presenting cells, as a result of the efficient uptake and processing of complexed autoantigens [17].

We have shown that the natural RF expressing B-lymphocyte subpopulation is hyperactive in RA twins irrespective of whether RA has developed. We hypothesize that this may arise as a result of inherited abnormalities in IgV regulation in pre-B cells. B-Lymphocytes derived from these progenitors could provide a fertile environment for generating signals which stimulate autoreactive T-lymphocytes. In turn, activated T-lymphocytes may promote mutations in B-lymphocytes and result in pathogenic RF generation. Thus a B-lymphocyte abnormality could correlate with HLA polymorphic genes, such as HLA-DR4/1, to initiate chronic RA with elevated RF levels. Demonstration of abnormal pre-B cells akin to those described in (NZB×W) F1 hybrids will be a logical next step in unravelling the multifactorial aetio-pathogenesis of RA [18].

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References
It is just over 2 years since the Editor last reviewed the Journal's progress [1]. Recent and forthcoming events necessitate the need once more to inform the readership of some changes and future plans. All major rheumatological journals continue to receive increasing numbers of original papers, both scientific and clinical, in spite of the recent proliferation of new journals, each devoted to a single disease. It seems likely that the major international journals will continue to prosper, most authors preferring their wider circulation both to individuals and to libraries which allows greater exposure of original papers, even when the subject matter is restricted to a single disease for which a specific journal is now also available.

Against this background, a modest increase in subscriptions for members of the British Society for Rheumatology has allowed us to increase the number of pages in each issue of the Journal up to 104 when necessary from the start of this year. The additional space will allow publication of more educational features. Some of these were postponed for lack of space. In parallel, the backlog of papers accepted but awaiting publication, unexpectedly created when submissions to the Journal almost doubled as we cautiously moved to monthly issues 2 years ago, has now been cleared. A strict audit of assessors and an even closer collaboration with our publisher now allows an assessment period of approximately 5 weeks and a period between acceptance and publication that would not normally exceed 4 months. We hope this improved service to authors will continue to attract high quality material, the volume of which is now sufficient to allow us to divide original papers into separate sections of the Journal slanted towards scientific research and clinical practice respectively, as a convenience to readers.

The acceptance of original manuscripts in computer disc format which in turn will further improve the efficiency of the service to authors as well as the turnover time is planned. Meanwhile, the Editor is always willing to consider any paper for 'fast tracking' upon the presentation of an appropriate argument in a covering letter when the paper is first submitted.

The steady climb in the position of the Journal in the scientific citation indices has been encouraging, assisted by the confidence of the British Society for Rheumatology in allowing us to publish their abstracts supplement regularly. The steady expansion of the Society ensures that this contains high quality original recent research that is likely to be cited. A steady increase in visitors from abroad at the British Society for Rheumatology meetings is also reflected by many international submissions to the Journal to cope with which our panel of international assessors has had to be expanded. We hope the Journal will continue to serve this international role, not just in respect of Europe and the Americas but also in respect of the expanding interest in rheumatology in the Far East and Africa.

Our international subscribers will have realized that clinical medicine in the United Kingdom is currently in a state of rapid flux. Health Service reforms introduced by the Government in the last few years that allow hospitals to acquire Trust status have introduced business-like principles, driven by consumer demand. This in conjunction with the new found authority of developing trusts, has prompted rapid change. In our own case, plans to re-site the hospital from which the Journal is edited closer to its parent University and our main centre of population, first proposed in 1956 to be completed by 1959, but which then lay fallow for 30 years, have been resuscitated and accomplished with almost frightening rapidity in a period of 15 months. As a result the address of the Editorial Office will change on the 23rd of March 1994 from Harrogate to Leeds. Full details of the new address, telephone and fax numbers will be published in our next issue as soon as they become available but arrangements will also be made to guarantee that mail sent to the old address is automatically redirected. The inconvenience of the move is