The Molecular Biology of Autoimmune Disease

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PREFACE

This publication represents the proceedings of a NATO Advanced Research Workshop which took place over 5 days in Athens, Greece in April of 1989. The aims of the meeting were to (i) highlight developments, particularly through recombinant DNA technology, in our understanding of the immune response and (ii) examine the implications of this new knowledge for autoimmunity and autoimmune disease.

The meeting was held as a Workshop of the 23rd Annual Scientific Meeting of the European Society for Clinical Investigation (ESCI). Sponsorship of the meeting, particularly from the NATO International Scientific Exchange Programme, but also from ESCI itself, Pharmacia Therapeutics AB (Sweden), Ortho Pharmaceutical Corporation (USA) and Napp Laboratories (UK) is gratefully acknowledged. In creating the scientific programme for the Workshop, Andy Demaine and I were helped enormously by our co-organisers Charles Janeway, Bernard Malissen, Terence Rabbitts and George Eisenbarth and their help too is gratefully acknowledged. A meeting and the resultant publication are only as good as those that contribute to them, both in terms of the scientific content of their presentation and the efficiency with which they then provide a manuscript for publication. In both senses, our contributors have been exemplary. I hope very much that the quality of their presentation and the enthusiasm which these generated will come over to the reader in the discussions

which followed their presentations. In editing the book Andy and I were helped by our colleague, Paul Banga, and we thank him. Through lack of space, one of the highlights of our meeting does not appear in this publication and that was the abstracts for the Poster Sessions. The quality of the abstracts submitted was high and they attracted considerable discussion. For those wishing to see a record of these abstracts they can be found in the European Journal of Clinical Investigation in Part II of Volume 19, No. 2 - April 1989. The burden of making sense of the recorded discussions of the various papers and the typing and retyping of the discussion and of unsatisfactory manuscripts fell to Jacqui De Groote in the Department of Medicine, and she has responded magnificently. David Ewins from the Department ensured we captured the images of our invited speakers on film, and even if we have had to censor some of his creations to ensure the speakers retain their images, his contribution is gratefully acknowledged! Finally, and most importantly, I wish to thank Christine Edwards, also in the Department, whose direction of the Directors, organisation of the Organisers, persuasion of authors to produce manuscripts and running of the actual Workshop in Athens and every other odd job associated with the Workshop ensured its outstanding success. I am particularly indebted to her.

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C O N T E N T S

ORGANIZATION AND EXPRESSION OF HUMAN V_H GENES

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Introduction

The variable regions of immunoglobulin heavy chains are encoded by the three germline gene segments: V(ariable), D(iversity) and J(oining). These segments are joined during precursor B cell differentiation to form a functional V_HDJ_H variable region gene. In the mouse there are hundreds of different V_H gene segments that can be subdivided into families based on nucleotide sequence homology (reviewed in Alt, et al., 1987). Various studies of transformed and normal murine B lineage cells have shown that chromosomal position of V_H segments is a major determinant of their rearrangement frequency, resulting in a preferential rearrangement of V_H segments proximal to the cluster of J_H elements (Yancopoulos, et al., 1984, 1988; Reth, et al., 1986; Perlmutter, et al., 1985). This preferential rearrangement phenomenon leads to the biased expression of J_H-proximal V_H segments in primary B cell repertoires; for example, these gene segments are the major contributors to the Ig heavy chain mRNA produced by B lineage cells of the fetal liver (Yancopoulos, et al., 1988). In contrast, B cells in peripheral lymphoid organs of adult mice appear to utilize most V_H segments at equal frequency; that is the representation of different families in the peripheral Ig heavy chain mRNA repertoire correlates with the complexity of each family and is not related to chromosomal position (Yancopoulos, et al., 1988; Dildrop, et al., 1985). These findings led to the proposal that an initially biased repertoire is randomized, probably by cellular mechanisms, in the transition from primary to peripheral lymphoid tissues (Yancopoulos, et al., 1988).

Although the potential significance of the biased primary repertoire is not clear, it has been suggested that proximal V_H genes may have evolved specificities important early in development for the establishment of the repertoire (Rajewsky et al., 1987; Holmberg 1987). One way to

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further analyze the significance of the preferential V_H gene rearrangement observed in mice is to determine whether similar phenomena exist in other mammalian species and, if so, to determine the nature of the antibodies encoded by proximal V_H genes. Recently, the general structure of the human Ig heavy chain variable region locus has been elucidated (Berman, et al., 1987; Kodaira et al., 1986). To begin to address the issues outlined above, we have studied the expression of V_H genes at different time points in B cell ontogeny and have correlated expression of the most J_H-proximal V_H segment with certain autoantibody specificities.

Organization of the human immunoglobulin heavy chain V_H locus

The human V_H locus contains 100-200 gene segments that have been grouped into 6 families (denoted V_H1-V_H6) that range in size from 1 member (V_H6) to more than 25 members (V_H3) (Berman, et al., 1987; Kodaira et al., 1986). In contrast to murine V_H organization patterns, members of the human V_H families are highly interspersed over the entire 2000 kb locus (fig. 1). We have employed pulsed field gel electrophoresis to demonstrate that the V_H locus begins within less than 90 kb of the J_H-$C\mu$ region with the V_H6 gene (a single membered family) being the most J_H-proximal human V_H gene segment (Berman, et al., 1987).

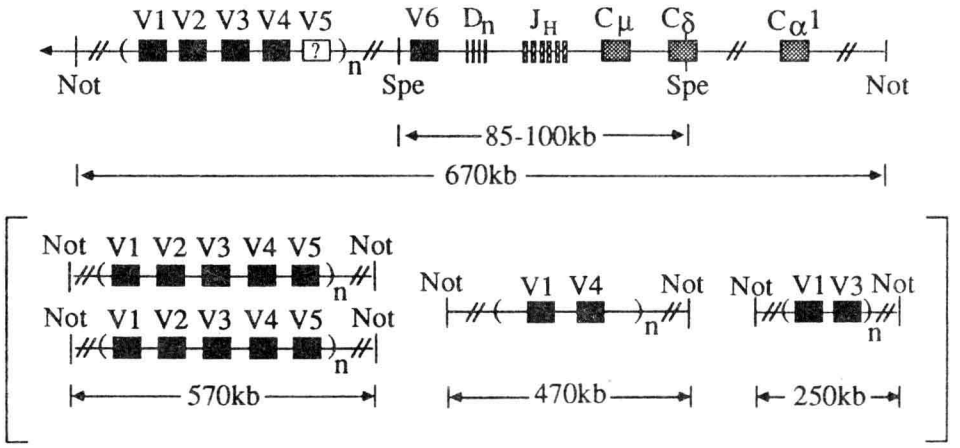

Figure 1. Organization of the human immunoglobulin V_H locus as determined by Pulse field gradient gel electrophoresis; for details see Berman et al., 1987.

Expression of Human V_H Genes in Primary and Peripheral Lymphoid Tissues

As an initial approach to assay the utilization frequency of V_H gene segments by surface Ig-positive B lymphocytes, we assayed the V_H family expressed by individual members of a collection of 187 monoclonal, IgM-secreting Epstein Barr virus (EBV)-transformed cell lines derived from adult and fetal tissues. The frequency of V_H family utilization by these lines roughly correlated with the complexity of the family (Table 1)--suggesting that the repertoire is randomized in the B lymphocytes that are the targets of EBV transformation. These results are reminiscent of those obtained with murine splenic hybridomas in which V_H utilization again correlated with family size.

	Tissue				
	adult periph. blood	19 week fetus liver	spleen	Total	Complexity no. of bands (%)
	(n=97)	(n=36)	(n=54)	(n=187)	
VH1	15	25	20	20	20-25 (33)
VH2	<5	<5	5	5	5-10 (11)
VH3	60	50	60	55	25-30 (40)
VH4	20	10	10	15	6-10 (11)
VH5	5	<5	10	5	2-3 (4)
VH6	<5	<5	<5	<5	1 (1)

Table 1. Frequency of V_H Gene Utilization in EBV-transformed Cell Lines. Total RNA from 187 monoclonal IgM-secreting EBV-transformed cell lines was analyzed in northern blotting experiments for hybridization to probes specific for each of the 6 V_H gene families. The data are presented as the percentage of the total number (n) of cell lines in a given collection that hybridizes to a V_H-specific probe.

To assay for the relative utilization of V_H gene families in primary and peripheral lymphoid cells, we used the Northern blotting assay previously described (Yancopoulos, et al., 1988). Briefly, in this assay, a standardized amount of Ig μ heavy chain mRNA is assayed for

hybridization to family-specific V_H probes; the ratio of hybridization of each V_H to RNA from a primary lymphoid organ (in this case 16 and 24 week old fetal liver) to that of a peripheral organ (in this case, T cell-depleted peripheral blood; PBL) is analyzed. The data from this preliminary experiment are shown in figure 2.

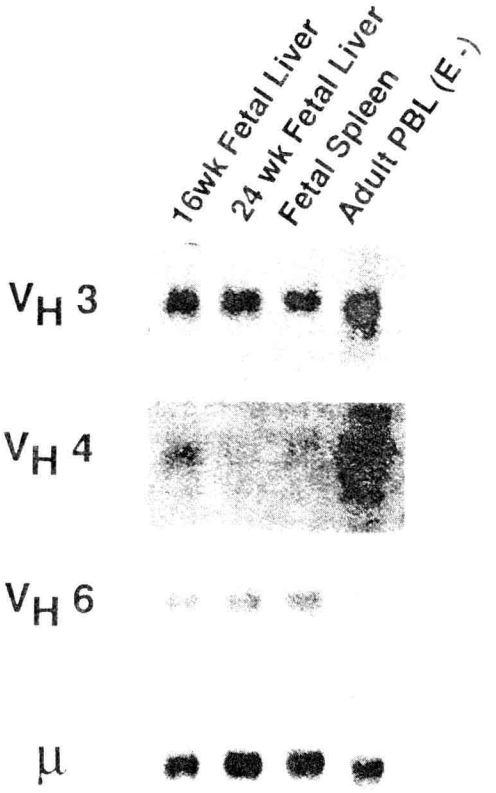

Figure 2. Biased V_H6 Gene Utilization in Early Development. RNA from two fetal livers (Lane 1 and 2), a fetal spleen (Lane 3) and adult peripheral blood non-T cells (Lane 4) was analyzed in Northern blotting experiments for hybridization to a V_H3, V_H4, V_H6 and $C\mu$-specific probe.

The V_H6 gene clearly hybridized much more intensely to the same amount of μmRNA from the fetal liver samples than to that from adult PBL. This result is very similar to that observed when the J_H proximal murine V_H81X gene is used to probe an equivalent amount of μmRNA from murine fetal liver and adult spleen (Yancopoulos, et al., 1988) and indicates that--like V_H81X in the mouse--the J_H proximal V_H6 gene

segment in humans is much more abundantly represented in the fetal liver as opposed to the PBL repertoires. As opposed to V_H6, the V_H3 family appears to be equally represented in the fetal and adult repertoires, while the V_H4 family appears to be more abundantly expressed in adult repertoires. The findings with the V_H3 family are consistent with our previous observations that this is the largest gene family and that its members are interspersed across the entire V_H locus; therefore representation changes based on either rearrangement or cellular selection would be expected to be less apparent at the gross level of this assay (Yancopoulos, et al., 1988); but could be more obvious if individual members of the family were examined (Schroeder, et al., 1987; see below). In the context of our previous findings in the murine system, the increased representation of the intermediate sized V_H4 family in PBL as compared to fetal liver could be explained in various ways including a large number of functional members and or a more distal location (Yancopoulos, et al., 1988). Notably, we find that V_H utilization patterns in 24 week fetal spleen are similar to those of fetal liver (Figure 2); we have made similar findings in the mouse (Malynn, et al., submitted), and various analyses suggest that the bulk of the mRNA detected in these tissues at this stage may come from immature B lineage cells.

Together, the results of our analysis of V_H gene utilization in human B lineage cells are quite analogous to those obtained by similar studies of the murine system. These findings suggest that an initially position dependent repertoire is generated in differentiating human pre-B cells and that this repertoire is normalized in surface IgM-positive B lymphocytes. This normalization was evident even in our analyses of EBV-transformed B cells from 19 week old fetal tissues (Table 1), suggesting that normalization processes may act rapidly on the newly formed, sIgM-positive B cells. As noted above, none of our analyses permit detection of qualitative differences between the collections of V_H genes expressed from multi-membered families. In this regard, others found preferential expression of particular V_H1 and V_H3 genes in a cDNA library of 19 week old fetal liver (Schroeder, et al., 1987). It should be noted that the organization of families appears somewhat different in humans versus the mouse. Murine families tend to be relatively clustered as units whereas human families are highly interspersed. It will be of interest to determine if the V_H3 genes

preferentially expressed in human fetal liver represent J_H-proximal members of that family.

Autoantibodies Encoded by the V_H6 Gene Family

Antigen-binding properties of IgM molecules secreted by the 187 EBV lines were assayed by screening culture supernatants in direct binding ELISA for reactivity against a panel of 24 antigens. A number of antibodies that employed heavy chains from families 1-5 bound to particular antigens within the panel; however, there was no obvious correlation between antigen-binding pattern and expression of these V_H families (not shown). In contrast, antibodies from each of the 4 V_H6-expressing cell lines in the collection bound to ssDNA and poly dT, regardless of the light chain expressed. In addition, V_H6 antibodies from individual cell lines displayed various patterns of binding activities with a limited number of antigens in the panel, notably dsDNA, cardiolipin, cytochrome C and hen egg lysozyme. Such polyreactivity is a characteristic of some monoclonal anti-DNA antibodies of both human and murine origin.

Clone #	origin	isotype	μg/ml of Ag required for 50% inhibition					
			ssDNA	nDNA	Card	PdT	CytC	Hel
A10[1]	adult PB	IgM,κ	5.0	0.02	0.1	0.02	-	0.02
A431[1]	adult PB	IgM,λ	5.0	0.3	0.2	5.0	2.0	-
L16[1]	130 d FL	IgM,λ	5.0	-	0.13	0.001	-	-
ML1[2]	130 d FS	IgM,κ	2.5	-	-	10.0	-	-

Table 2. Origin of V_H6-expressing EBV-transformed B Cell Lines and Properties of the Antibodies they Secrete. Liver and spleen cells from a 19 week old fetus and mononuclear peripheral blood cells from 3 adults were transformed with EBV under limiting dilution conditions. Supernatants from monoclonal, IgM-secreting lines were tested for reactivity against a panel of 24 antigens including haptens, protein (auto)antigens, polysaccharides and polynucleotides. Results obtained from the direct binding assays were confirmed in liquid phase inhibition ELISAs. Results are expressed as the concentration of competitor required for 50% inhibition of binding to solid-phase bound antigen. PB: peripheral blood. FL: fetal liver. FS: fetal spleen.