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Dynamics of the reactivity to MBP in multiple sclerosis

Antonio Uccelli*[,1], Giovanni Ristori[3], Debora Giunti[1], Marco Seri[2], Chiara Montesperelli[3], Francesco Caroli[2], Claudio Solaro[1], Alessandra Murialdo[1], Monica Marchese[1], Carla Buttinelli[3], Gianluigi Mancardi[1] and Marco Salvetti[3]

[1]Department of Neurological Sciences and Vision, University of Genoa, Via De Toni 5, 16132 Genoa, Italy; [2]Molecular Genetics Unit, Gaslini Institute, Genoa, Italy and [3]Department of Neurology, La Sapienza University, Rome, Italy

Though many lines of evidence support the importance of myelin basic protein (MBP) in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), its role in multiple sclerosis (MS) is still debated as well as the significance of epitope spreading in disease progression. We characterised the response to MBP in eight MS subjects and three of these were followed over time. In one case, the follow up lasted over a 6-year period. Clonal expansion, clonal persistence and epitope spreading against other MBP determinants was detected irrespective of disease course. In one patient we identified a novel T-cell receptor variable gene (BV28S2) which may be involved in the selection of MBP determinants, as suggested by experiments performed in the presence of mismatched antigen presenting cells (APC) between two subjects compatible for HLA-DR2 subtype but differing for the epitope recognised. Our findings do not sustain a role for the response to MBP effecting on clinical course and suggest that a novel TCR gene may be involved in the recognition of unusual self antigens. *Journal of NeuroVirology* (2000) **6,** S52–S56.

Keywords: multiple sclerosis; myelin basic protein; T-cell receptor; experimental autoimmune encephalomyelitis

Introduction

Myelin basic protein (MBP) is capable of inducing experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis (MS), in susceptible species (Lassman and Wekerle, 1998). In many species MBP-specific T-cells are part of the mature repertoire but they are not capable of inducing disease unless properly triggered (Genain *et al*, 1994). Upon immunisation with MBP, animals develop a vigorous response to the immunogenic protein, which in the early phases of disease is restricted in terms of specificity and T-cell receptor (TCR) usage (Zamvil and Steinman, 1990). Progression of disease is characterized by the recruitment of T-cells specific for an increasingly broad set of self determinants, phenomenon called 'epitope spreading' (Lehmann *et al*, 1993; Yu *et al*, 1996). Although the response to MBP in humans has been dissected in detail by scientists over the last 10 years, its role in MS pathogenesis still lacks definitive evidence. With this in mind, we studied the response to MBP in a group of eight MS patients. While in most individuals we generated T-cell lines (TCL) recognizing multiple MBP determinants, in three patients with mild disease the response was focused only against a single epitope. After 1 year no clinical changes were observed and in two out of three subjects we detected clonal persistence of T-cell specific for the same epitope. In one subject, followed for another 5 years, we observed spreading of the response against other MBP determinants in the absence of disease progression. In the same individual we identified a novel TCR β chain variable gene (BV28S2) which may be involved in the preferential recognition of an unusual MBP determinant (aa 16–38).

*Correspondence: A Uccelli

Results

Dynamics of the MBP-specific repertoire

At T1, TCL recognised three immunodominant epitope of MBP, namely aa 16–38, 80–104 and 142–172. In three subjects the response was restricted to a single determinant (not shown). In one subject clonal expansion of MBP specific T-cells was sustained by the presence of two TCL

carrying the same rearrangement (Table 1). At T2, after 1 year, some TCL recognized the same epitope 80–104 but expressed a different TCR Vβ rearrangement. Nevertheless, one line still utilized the identical complementary determining region 3 (CDR3) observed at T1 suggesting clonal persistence. Clonal persistence of T-cells specific for aa 16–38 was observed also in a second patient, in which the response after 1 year was still focused against the same epitope (16–38) (Table 1). TCL established in the same patient at the third and the fourth time points (T3, T4) responded to several other epitopes, particularly in the C-terminal region of MBP (data not shown). The TCR repertoire changed accordingly. Clonal persistence of T-cells specific for the peptide 108–131 was observed between T3 and T4 while clonal expansion of aa 16–38 specific T-cells was detected at T3 (Table 1). The above dynamics of the MBP-specific T cell repertoire did not correlate with changes in disease progression, since all four patients, particularly the subject with the longest follow up, had a stable and benign disease irrespective of the occurrence of epitope spreading and clonal expansion of T-cells with different specificity.

Antigen processing and presentation by mismatched APC of DR compatible individuals

Compatibility for antigen processing was studied in proliferation assay with mismatched APC utilizing TCL from two individuals, HLA-DR compatible, recognising different determinants, namely aa 16–38 in one subject and 86–99 in the other, as well as the whole MBP (Figure 1). Effective proliferative responses were observed also when the antigens were pulsed on the APC in serum-free condition, thus excluding processing outside the endocytic pathway or mutual presentation by activated T cells. Proliferation against the relevant antigen but not against MBP was obtained in the presence of fixed (both autologous and compatible) APC, confirming the compatibility for presentation and the need for MBP processing (Figure 1).

Identification of a novel TCR gene

Interestingly, two TCL, obtained from the individual whose TCL recognized aa 16–38, expressed an identical TCRBV gene whose sequence did not show similarity >75% with any functional BV gene described thus far at the cDNA level. On-line comparison of nucleotide and corresponding amino acid sequences of this TCR segment with those in nucleotides and amino acids databases revealed a high degree of similarity (92%) only with a

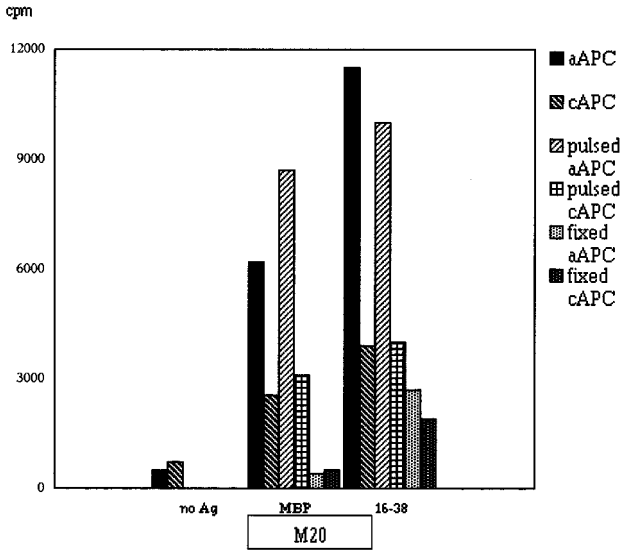

Figure 1 Proliferative response to MBP and to 16–38 of a TCL (M20) assayed in the presence of (1) autologous APC (aAPC); (2) APC from a DR compatible donor (cAPC) whose T-cell response focused against a different epitope (86–99); (3) APC pulsed in serum free conditions; and (4) fixed APC.

Table 1 CDR3 junctional regions of TCL from two MS subjects, PA and SA, at different time points

Subject	*TCL*	*Gene usage*	*CDR3 sequence*	*Time*	*Epitope*
PA	M7	BV28S2 BJ2S6	RSGGANVLT	T1	16–38
PA	M20	BV28S2 BJ2S6	RSGGANVLT	T2	16–38
PA	M7	BV21S1 BJ1S2	SLAHVRGGYGYT	T1	16–38
PA	M20	BV21S1 BJ1S2	SLAHVRGGYGYT	T2	16–38
PA	M29	BV13S6 BJ2S1	SPAGGEQF	T3	16–38
PA	M31	BV13S6 BJ2S1	SPAGGEQF	T3	16–38
PA	M33	BV12S1 BJ2S1	ESRSSYNEQF	T3	108–131
PA	M16	BV12S1 BJ2S2	ESRSSYNEQF	T4	108–131
SA	7	BV13S6 BJ2S2	YGGLGGELF	T1	80–104
SA	20	BV13S6 BJ2S2	YGGLGGELF	T1	80–104
SA	H10	BV13S6 BJ2S2	YGGLGGELF	T2	80–104
SA	14	BV21S2 BJ1S1	RSDRGAPEAF	T1	80–104
SA	E12	BV21S2 BJ1S1	RSDRGAPEAF	T2	80–104

Clonal persistence and clonal expansion were defined as presence of TCL carrying identical CDR3 raised at distinct time points, and of TCL carrying identical DCR3 individually obtained at the same time point, respectively.

sequence retrieved from GenBank (accession number L36092) and representing a pseudo-gene (BV28S1) located within the 685 Kb TCR locus (Figure 2).

Discussion

Although we and others have detected the presence of clonally expanded MBP-specific T-cells (Uccelli *et al*, 1998; Wucherpfenning *et al*, 1994; Vandervyver *et al*, 1995) as part of a complex and heterogenous set of self-reacting T-cells, their role in MS pathogenesis is still debated. Our results demonstrated the presence of clonal expansion, clonal persistence and intra-molecular epitope spreading of MBP-specific T-cell populations within the peripheral compartment of MS patients at different time points. Epitope spreading was confirmed by the spreading of the TCR repertoire as well. Three patients were followed for 1 year and a fourth over a 6-year period. In no individual did we observe a correlation between such dynamical changes of the antigen specific T-cell response and clinical course, as all patients were characterised by a stable and benign disease. Genetic background and mechanisms of degenerate cross-reactivity between self and foreign antigens may account for these ephemeral phenomena (Hemmer *et al*, 1997). As a degenerated recognition of antigens by the TCR seems to be a feature of the immune system, it is likely that concurrent factors are required to shift the natural cross-recognition to frank autoimmunity (Gran *et al*, 1999). Previous works reported that chronic progression of EAE is associated with the spreading of responses to new determinants (Lehmann *et al*, 1993; Yu *et al*, 1996). Recently, it has been shown that disappearance of early autoreactivity to determinants within the proteolipid protein (PLP) and shifting to new target epitopes in patients with isolated demyelinating syndromes correlates with chronic progression to clinically definite MS (Tuohy *et al*, 1999). Our data do not confirm that epitope spreading to other self-determinants correlates with disease progression. This could be explained by the non-relevance of the T-cell response to MBP in the pathogenesis of demyelinating diseases in outbred primates, as suggested by the lack of encephalitogenicity of MBP in marmosets (Uccelli, unpublished results). Alternatively, it is possible that the recruitment of the T cell with additional specificities involves also lymphocytes with protective functions, as recently demonstrated in EAE (Kumar, 1998). These findings support the hypothesis of an 'epitope du jour' suggested by Tuohy and colleagues based on the concept that immunodominant responses against self-antigens change with time (Tuohy *et al*, 1999) and underline the importance of a sufficiently long follow-up in serial studies on determinant spreading. We also showed that TCR may, in some instances, play a role in determining the immunodominance of MBP determinants. This is supported by the recognition of an unusual epitope, aa 16–38, only by TCL carrying a TCV Vβ gene, BV28S2, yet undescribed. Conversely, the same TCL recognised both the whole MBP and the relevant peptide also in the presence of non-autologous APC from a DR compatible donor, thus excluding that differences in the processing machinery could be responsible for the selection of the immunodominant epitope (Ristori *et al*, 1999).

BV28S2 must be a rarely expressed TCR element, since it was never isolated either by random cloning strategies or from the analysis of antigen specific T-cells and, more important, does not lie within the TCRβ chain locus, recently described (Rowen *et al*, 1996). The identification of a novel TCR gene expressed by TCL specific for a self antigen demonstrates that BV28S2 is functional and part of the human peripheral repertoire. Interestingly, this gene was used by some MBP-specific TCL obtained from marmosets immunised with whole myelin (Uccelli, unpublished data). Thus, this new

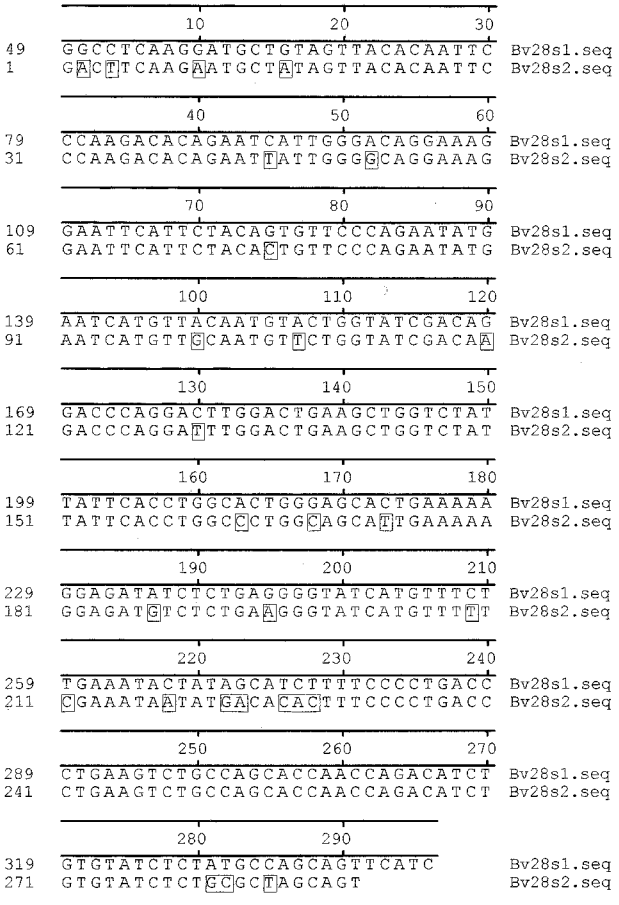

Figure 2 Alignment of BV28S2 and BV28S1 nucleotide sequences. The box indicates a mismatch.

family, although rarely expressed, may have an important role in the response to putative autoantigens.

Materials and methods

At the first time point (T1), we obtained TCL from eight patients with relapsing remitting MS. TCL were established at a second time point (T2) only from those individuals who recognised a single MBP determinant at T1. In one subject, TCL were obtained at two other time points over a 6-year period. MBP specific TCL were established using the 'split-well' technique and proliferation assay and epitope mapping were performed as previously described (Salvetti *et al*, 1993).

The role of antigen processing and presentation in the selection of the MBP deteminants was evaluated in proliferation assays in which antigen presenting cells (APC) from two MS patients sharing the DRB1*1501, DRB5*0101 haplotype, but differing for the presence of restricted responses to distinct MBP regions, had been reciprocally mismatched. The same protocol was performed in serum-free pulsing experiments, or in the presence of 'fixed' APC to exclude extracellular processing or mutual T cell presentation as described elsewhere in detail (Ristori *et al*, 1999).

Analysis of TCR usage of TCL was performed by RT–PCR with a panel primers specific for 24 Vβ and in some case by inverse-PCR as previously reported (Uccelli *et al*, 1998). Analysis of transcripts was carried out using the software Lasergene for Windows (DNASTAR Inc., Madison, WI, US) and TCR sequences were compared with Genbank databases.

The sequence of the novel TCR gene reported in this paper has been deposited in GenBank under accession number AF064083.

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References

Genain CP, Lee-Parritz D, Nguyen MH, Massacesi L, Joshi N, Ferrante R, Hoffman K, Moseley M, Letvin NL, Hauser SL (1994). In healthy primate, circulating autoreactive T cells mediate autoimmune disease. *J Clin Invest* **94:** 1339–1345.

Gran B, Hemmer B, Vergelli M, McFarland HF, Martin R (1999). Molecular mimicry and multiple sclerosis: degenerate T cell recognition and the induction of autoimmunity. *Ann Neurol* **45:** 559–567.

Hemmer B, Fleckenstein B, Vergelli M, Jung G, McFarland H, Martin R, Wiesmuller KH (1997). Identification of high potency microbial and self ligands for a human autoreactive class II restricted T-cell clone. *J Exp Med* **185:** 1651–1659.

Kumar V (1998). Determinant spreading during experimental autoimmune encephalomyelitis: is it potentiating, protecting or participating in the disease? *Immunol Rev* **165:** 73–80.

Lassmann H, Wekerle H (1998). Experimental models of multiple sclerosis. In: *McAlpine's multiple sclerosis*. Compston A, Ebers G, Lassmann H, McDonald I, Matthews B, Wekerle H (eds) Churchill Livingstone: London, Edinburgh, New York, Philadelphia, Sidney, Toronto, pp 409–433.

Lehmann PV, Sercarz EE, Forsthuber TG, Dayan CM, Gammon G (1993). Determinant spreading and the dynamics of the autoimmunity T-cell repertoire. *Immunol Today* **14:** 155–157.

Ristori G, Montesperelli C, Uccelli A, Giunti D, Buttinelli C, Bomprezzi R, Mancardi GL, Salvetti M (1999). A major influence of the T cell receptor repertoire as compared to antigen processing/presentation in the selection of myelin basic protein epitopes in multiple sclerosis. *J Neuroimmunol* **96:** 241–244.

Rowen L, Koop BF, Hood L (1996). The complete 685-kilobase DNA sequence of the human beta T cell receptor locus. *Science* **272:** 1755–1762.

Salvetti M, Ristori G, D'Amato M, Buttinelli C, Falcone M, Fieschi C, Wekerle H, Pozzilli C (1993). Predominant and stable T cell responses to regions of myelin basic protein can be detected in individual patients with multiple sclerosis. *Eur J Immunol* **23:** 1232–1239.

Tuohy VK, Yu M, Kawczac JA, Kinkel RP (1999). Spontaneous regression of primary autoreactivity during chronic progression of experimental autoimmune encephalomyelitis and multiple sclerosis. *J Exp Med* **189:** 1033–1042.

Uccelli A, Giunti D, Salvetti M, Ristori G, Fenoglio D, Abruzzese M, Mancardi GL (1998). A restricted T cell response to myelin basic protein is stable in multiple sclerosis patients. *Clin Exp Immunol* **111:** 186–192.

Wucherpfenning KW, Zhang J, Witek C, Matsui M, Modabber Y, Ota K, Hafler DA (1994). Clonal expansion and persistence of human T cells specific for an immunodominant myelin basis protein peptide. *J Immunol* **152:** 5581–5591.

Vandevyver C, Mertens N, Van den Elsen P, Medaer R, Raus J, Zhang J (1995). Clonal expansion of myelin basic protein-reactive T-cells in patients with multiple scler-osis: restricted T-cell receptor V gene rearrangements and CDR3 sequence. *Eur J Immunol* **25:** 958–968.

Yu M, Johnson JM, Tuohy VK (1996). A predictable sequential determinant spreading cascade invariably accompanies progression of experimental autoimmune encephalomyelitis: a basic for peptide specific therapy after the onset of clinical disease. *J Exp Med* **183:** 1777–1788.

Zamvil SS, Steinman L (1990). The T lymphocyte in experimental allergic encephalomyelitis. *Ann Rev Immunol* **8:** 579–621.