Familial aggregation of polymyalgia rheumatica and giant cell arteritis: Genetic and T cell repertoire analysis

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Abstract

Objective
Several reports of familial aggregation of giant cell arteritis (GCA) and polymyalgia rheumatica (PMR) have been described although detailed genetic and immunological studies are scarce. Our aims were to investigate the influence of HLA-DRB1 alleles and to analyze the phenotype and T cell receptor (TCR) usage of circulating T lymphocytes in a familial case of GCA and PMR.

Methods
HLA-DRB1 typing was carried out using polymerase chain reaction amplification with specific primers. The study of the circulating T cell repertoire was performed by staining with specific monoclonal antibodies and flow cytometry analysis.

Results
Patient 1 developed GCA at the age of 71, four years prior to the diagnosis of PMR in her older brother. The HLA-DRB1 typing of Patient 1 was DRB1*04 (DRB1*0401)/DRB1*12 and in Patient 2 was DRB1*07/DRB1*12. In our patient population, GCA was associated with an increased frequency of HLA-DRB1*04 compared with PMR patients. Regarding T cell phenotype, the brother with active PMR had a higher expression of surface markers indicating activation in both T cell subsets (CD25 and HLA-DR). The sister with GCA showed a pronounced decrease of CD4+/CD45RA+ T cells with respect to her brother with PMR. Both patients carried a significant depletion of CD28 in both subsets, specially within the CD8+ T cell compartment. The BV gene usage differed from one patient to the other. T cell expansions were identified in both patients but the specificities were different.

Conclusion
We describe an association of GCA and PMR between two first degree relatives with significant genetic and immunologic differences. Our results suggest that the pathogenic mechanisms leading to the development of GCA and PMR are probably multifactorial, and both genetic and environmental factors may contribute to the development of these diseases.

Key words
Polymyalgia rheumatica, giant cell arteritis, HLA, T lymphocytes.
Familial aggregation of PMR and GCA / M.J. Bartolome et al.

Introduction

The etiopathogenesis of giant cell arteritis (GCA) and the closely related syndrome polymyalgia rheumatica (PMR) remains unknown although genetic, autoimmune, and environmental factors have been implicated (1, 2). Considerable evidence points to a genetic predisposition in both syndromes: a) they are more frequent in women (1, 3); b) both syndromes are more frequent in Caucasians (especially in the countries of northern Europe and in some regions of the USA with a high proportion of residents of Scandinavian ancestry), and rarely occur in African or Asian populations (1, 4, 5); c) HLA typing studies have shown a consistent association of both syndromes with certain alleles of the HLA-DRB1 molecule in different populations (6-9); and finally d) several reports of a familial aggregation of GCA and PMR have been also described (10-22).

We present a new familial case of PMR and GCA and describe the phenotype and T cell receptor (TCR) usage of circulating T lymphocytes together with HLA-DRB1 typing. The possible pathogenetic mechanisms for this familial association are also discussed.

Patients and methods

Patient 1

A 71-year-old woman presented to our hospital in October 1994 because of a 3-month history of malaise, weight loss, sore throat, and fever. She denied headache, jaw claudication or visual manifestations. On physical examination, she was febril (38.5°C), the erythrocyte sedimentation rate (ESR) was 120 mm/1 hour, and the C reactive protein was 1.3 mg/dl. Hemoglobin and hematocrit levels were decreased (10.3 g/dl and 32%, respectively), the erythrocyte sedimentation rate (ESR) was 120 mm/1 hour, and the C reactive protein was 1.3 mg/dl (normal range < 0.5 mg/dl). Thyroid function was within the normal range. The remaining imaging and laboratory studies were normal. Biopsy of the left temporal artery (specimen length: 3.5 cm) was normal. Despite the low ESR, and due to the suspicion of PMR (24), he was treated with prednisone 10 mg/day b.i.d, and an excellent clinical response was seen within 4 days with progressive recovery of weight and a return of C reactive protein to normal levels. After 20 months of follow-up he remains asymptomatic on 2.5 mg/day of prednisone.

Preparation of cells and HLA typing

Peripheral blood mononuclear cells (PBMC) were purified from EDTA venous blood by Ficoll gradient centrifugation (Boehringer Ingelheim, Heidelberg, Germany). DNA was extracted with DNAzol™ reagent (Life Technologies, Grand Island, NY). Both patients were characterized for their HLA-DRB1 alleles by allele specific...
PCR amplification with a Dynal All Set™ SSP and Dynal DRB1*04-SSP (Dynal AS, Oslo, Norway). The amplifications were performed in a thermocycler (Geneamp PCR System 9700, Perkin-Elmer) following the manufacturer instructions, and the amplified products were separated on a 2% agarose gel and stained with ethidium bromide.

**FACS analysis**

Peripheral blood T lymphocyte phenotype and TCR usage were determined by flow cytometry using a whole blood lysis technique with the following monoclonal antibodies (mAb): FITC-conjugated anti-CD3 (Becton Dickinson, San Jose, CA), -CD7, -CD45RA, -CD45RO, -HLA-DR (Pharmigen, San Diego, CA), -CD25, -CD28, -CD57, -BV2 (Labgen, Labclinics, Barcelona), -BV3S1, -BV5S1, -BV5S2/5S3, -BV6S7, -BV8, -BV13S1/13S3 (T Cell Diagnostics, Woburu, MA), -BV12 and -BV17 (Endogen, Woburu, MA), PE-conjugated anti-CD4 and -CD8 (Becton Dickinson). Whole blood samples were stained within 3 hours of venipuncture with two-color direct immunofluorescence reagents. Erythrocytes were lysed using FACS® Brand Lysing Solution (Becton Dickinson) and cells were analyzed on the day of processing using a Becton Dickinson FACScalibur and Simulset software. Twenty thousand events were acquired. T-cell expansion was defined as BV specificities expressed at a frequency greater than the mean +3 standard deviations (SD) of the young healthy donors or as a value of more than 20%.

**Results**

**Family history and HLA-DRB1 genotype analysis**

There are 4 brothers and 3 sisters in this family. All of them except for one sister live in the same town but in different houses. Their mother died at the age of 73 from a cerebrovascular event and their father died at the age of 65 due to gastric cancer. The sister and brother with GCA and PMR, respectively, had no recognized activities or other relevant past history that were not shared by the unaffected members of the family. No other first degree family member, spouses, or close friends had recognized GCA or PMR or other vasculitic disorders.

Patient 1 developed GCA at the age of 71, four years prior to the diagnosis of PMR in her older brother. Although the clinical presentation at the time of diagnosis was not classical for GCA, the diagnosis was based on a temporal artery biopsy which showed the typical findings of arteritis (23). Furthermore, she responded promptly to high-dose corticosteroids and remains free of symptoms after a long-term follow-up. The HLA-DRB1 typing of Patient 1 was DRB1*04 (DRB1*0401) / DRB1*12.

Patient 2 developed a classic PMR syndrome at the age of 78 and although he lacked the typical increased ESR, the C reactive protein levels revealed an ongoing inflammatory process (24,25). He also responded rapidly and completely to low dose prednisone, and remains free of symptoms after more than one year of follow up. The HLA-DRB1 typing of Patient 2 was DRB1*07 / DRB1*12.

Since our two patients carried different HLA-DRB1 alleles, we next evaluated whether the genetic background might influence the development of these diseases. To this end we analyzed the HLA-DRB1 alleles in a cohort of 39 consecutive PMR and 21 GCA patients followed in our Division and compared the results with the distribution of the same allelic variants in 60 healthy controls. The HLA-DRB1 allelic distribution was very similar between PMR patients and controls. As shown in Figure 1, we did not find any significant difference in the distribution of DRB1*04, DRB1*07, and DRB1*12 between PMR patients and the control group. However, when we compared the distribution of the same alleles with the GCA group, DRB1*04 was significantly more frequent in GCA than in PMR (p = 0.049; Fisher exact test) and there was also a tendency for it to be more frequent than in healthy controls (p = 0.068). Although HLA-DRB1*07 was more frequent in healthy controls and PMR patients than in GCA, the difference did not reach statistical significance. The distribution of HLA-DRB1*12 was similar between the 3 groups.

**Peripheral T lymphocyte phenotype**

The circulating T lymphocyte phenotype was studied in both CD4+ and...
CD8+ T cells with a panel of 7 mAb (Table I). The analysis of the T cell phenotype between the two siblings disclosed several differences. As expected, the brother with active PMR had a higher expression of surface markers indicating activation of both T cell subsets (CD25 and HLA-DR). Within the CD4+ subset, both patients differed greatly in terms of the expression of the naive marker (CD45RA), and to a lesser extent the expression of CD7 and CD45RO. In particular, the sister with GCA showed a pronounced decrease in CD4+/CD45RA+ T cells with respect to her brother with PMR. Although both patients showed a significant depletion of CD4+/CD28+, and especially of CD8+/CD28+ T cells, there was not a significant difference between the two patients regarding the expression of the co-estimulatory molecule CD28.

Peripheral T lymphocyte TCR analysis

We analyzed the TCR repertoire of circulating T lymphocytes using 9 BV-specific mAb which account for approximately 40-50% of the total T cell repertoire (26). BV elements with frequencies exceeding the mean by at least 3 SD of young healthy donors or as a value by more than 20% were identified and defined as T cell expansion.

Following this definition we were able to identify T cell expanded populations in both patients (Fig. 2A and 2B). Four years after the diagnosis and when she was in clinical remission, the sister with GCA carried two expanded populations in the CD4+ subset (BV3S1 and BV17) and one T cell expansion within the CD8+ compartment (BV5S2/S3). At the initial evaluation and before steroid therapy, the brother with PMR also had expanded populations in both T cell subsets: a T cell expansion in CD4+/BV13S1+ T cells and in CD8+/BV8+ cells. It is important to note that a second analysis carried out in the patient with PMR two months after steroid therapy and when he was asymptomatic, revealed no significant changes in the distribution of T cell expansion (data not shown).

Discussion

This report describes an association of GCA and PMR between two siblings with significant genetic and immunologic differences. We examined the possible influence of these factors using PCR-based HLA-DRB1 oligotyping and flow cytometry analysis of the circulating T cell repertoire.

The family history of our patients did not show any previous cluster of vasculitic or related syndromes. However, the HLA-DRB1 genetic background in our cases was clearly different. As shown in Figure 1, in our patient population GCA is associated with an increased frequency of HLA-DRB1*04 compared with PMR patients. Consistent with this picture, the patient with GCA carried the HLA-DRB1*0401 allele which is one of the alleles most frequently associated to GCA in the literature (6). In contrast, the brother with PMR did not carry any HLA-DRB1*04 allele. This data suggests that the HLA-DRB1*04 allele might be important in the development of GCA, in disagreement with previous reports in which no predictive value for the development of systemic vasculitis was found for HLA-DRB1 alleles (8). Nevertheless, this data should be confirmed in a larger series of patients.

The HLA genes could affect disease pathogenesis through several mechanisms. One of the major biological functions of the HLA molecule is to bind the antigen with high specificity and present it to T cells, the so-called peptide selection model. In GCA, this model would be supported by the demonstration of the presence of selected CD4+ T cell populations expanded in the arterial wall that proliferate against inflamed temporal artery tissue extracts (27). HLA-DR polymorphisms may also shape the selection of the T cell repertoire and determine the severity of the manifestations of a disease, as has been demonstrated in rheumatoid arthritis patients (28). Unfortunately, and despite the relative high frequency of PMR and GCA, the influence of the HLA molecules on the diversity of the peripheral T cell repertoire in this disease has not been studied.

There is evidence that the circulating T cell compartment undergoes age-associated changes in the distribution of T cell subsets. In general, the number of naive T cells declines with age whereas the proportion of memory T cells increases. The expression of the co-stimulatory molecule CD28 also decreases with aging (29). In this age group, the decrease of the expression of CD28 has been considered by some authors as a terminal state of immune activation (30). The phenotype of circulating T lymphocytes in our 2 patients was com-

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<th>Time 0</th>
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<tr>
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<td>3.7</td>
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<td>CD4+ / CD28+</td>
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<tr>
<td>CD4+ / HLA-DR+</td>
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pared with the mean values of 21 age-matched healthy controls (Table I).

The most striking feature of the circulating T cell repertoire in our two patients was the decrease of CD28 expression in both T cell subsets, especially within the CD8+ compartment. Conversely, there was an increased frequency of CD57+ T cells in both patients with a slightly predominance in the PMR patient with untreated disease. Although normal aging is an important factor in the decrease of CD28 expression in circulating T lymphocytes, it is not the only determinant which correlates with the loss of CD28. Increased proportions of CD28- T lymphocytes have been also documented in diseases involving chronic antigenic stimulation (31). Chronic immune stimulation resulting in clonal exhaustion may contribute to immune dysfunction. This decrease of CD28 expression is more prominent within the CD8+ subset, which is the subset involved in viral immunity. Furthermore, there is clinical evidence that the control of certain latent viral infections is diminished in the elderly (31).

The severe depletion of CD28 expression on circulating T cells present in our two patients may contribute to the development of disease in two different ways. First, it could reflect a possible infective agent (such as a viral infection) as being responsible for these syndromes, a hypothesis that is supported by an increasing amount of epidemiologic, clinical and laboratory evidence (32). Second, and not mutually exclusive with the first hypothesis, is that the loss of expression of CD28 with aging may contribute to a state of immunodeficiency that may render a susceptible individual prone to the development of a disease (31).

Recent studies have demonstrated that the TCR repertoire of circulating T cells is not as extensive as previously thought (33, 34). Analysis with mAb and PCR-based techniques have demonstrated that circulating T cells include clonal populations, especially within the CD8+ subset (26, 33-35). The physiological consequences of this phenomenon are not completely understood, but it has been proposed that these clonal populations are the consequence of repetitive antigen exposure and might result in a reduced immunoresponsiveness (36). The TCR repertoire has been examined in patients with PMR and GCA in two recent studies (26, 37). In both of them PMR and GCA patients carried multiple expanded T cell populations, particularly within the CD8+ T cell subset.

In the light of these observations, it can be hypothesized that age-related changes in the TCR repertoire might predispose elderly subjects to the development of these syndromes (36). We therefore investigated the TCR VB repertoire in our two patients to see if there was any skewing. Although we demonstrated T cell expansion in both patients, the BV gene usage differed from one patient to the other. This data suggests that the TCR BV repertoire became oligoclonal in both siblings but that the specificities were different. These differences might be the result of the different stages of activity of the disease in the two patients. Alternatively, the differences in clinical expression

Fig. 2. Analysis of the T cell receptor (TCR) repertoire of circulating T lymphocytes in: (A) CD4+ T cells; and (B) in CD8+ T cells. A comparison of TCR BV usage between the GCA sister (white bars) and the PMR brother (grey bars) is shown. BV elements with frequencies exceeding the mean by at least three standard deviations of the young healthy donors or as a value of more than 20% were defined as T cell expansion. T cell expanded populations in both patients are identified by an asterisk (*). The results are expressed as percentages of each BV family.
between the two siblings might be explained by differences in the TCR repertoire observed. However, a second analysis carried out in the patient with PMR two months after steroid therapy, when he was asymptomatic, revealed no significant changes in the distribution of T cell expansion, suggesting that these expanded populations probably are not directly involved in the disease process. This finding has been previously reported in patients with these syndromes (26,37). The results of the present study suggest that the pathogenic mechanisms leading to the development of GCA and PMR are probably multifactorial, and that both genetic and environmental factors may contribute to the development of these diseases.

References