LETTERS

Multiple haematological malignancies in a patient with rheumatoid arthritis without exposure to disease modifying therapy

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Ann Rheum Dis 2006;**65**:268–269. doi: 10.1136/ard.2005.038596

Rheumatoid arthritis (RA) may be associated with an increased risk of haematological malignancy, perhaps as a result of RA itself or of its treatment. We describe the unusual case of a patient with longstanding RA who developed both lymphoproliferative and myeloproliferative malignancy without exposure to disease modifying drugs.

A 64 year old woman, wheelchair dependent with spina bifida related spastic paraparesis, had seropositive RA never treated with disease modifying drugs. In 1993, she developed Philadelphia chromosome positive, chronic myelogenous leukaemia (CML), treated with busulfan, initially 2 mg daily and subsequently on alternate days. Within 3 months, her total white cell count normalised and busulfan was discontinued. No further treatment was required for the CML.

Over subsequent years she required increasing support, including inpatient care, for immobility due to spina bifida, progressive RA, treated with low dose oral steroids with bone protection, and severe bilateral leg ulceration, crusted stasis dermatitis, and superficial pustules, treated with topical betamethasone valerate, bandaging, and support stockings.

In 2003, unusual erythematous soft tissue swellings were seen on her legs (fig 1) and scalp. Biopsy showed the dermis overrun with a diffuse population of large cells with a high mitotic rate, and a diagnosis of diffuse large B cell lymphoma was confirmed by detailed immunostaining. The patient died from bronchopneumonia shortly after the diagnosis was confirmed.

Increased haematological malignancy is well recognised in patients with RA, but there is disagreement about which subtypes occur. While an increased risk of leukaemia has not been a universal finding, increased lymphoproliferative malignancy in RA is more consistently described.¹ It has been postulated that persistent immune stimulation in RA may result in both chronic synovitis and persistent lymphocytic activation, with dysregulation of B cell proliferation, and, whether due to disease chronicity, aging, or treatment, eventual malignant transformation.² More recent reviews have discussed the role of high inflammatory disease activity, Epstein-Barr virus infection, and immunosuppressive treatment in the development of lymphoma.³ Methotrexate treatment may be a risk factor for lymphoma in patients with RA, although epidemiological studies do not support this: several case reports have described patients developing B cell non-Hodgkin's lymphoma where the lesions regressed after methotrexate treatment was stopped.^{4 5}

However, although RA treatment may play a part, there is evidence that RA itself may be associated with increased haematological malignancy. A study of the distribution of lymphoma subtypes in patients with RA who had received very little immunosuppression found an increased incidence of diffuse large B cell lymphoma (DLBCL).⁶ DLBCL, classified by the World Health Organisation under mature B cell neoplasms, is a rare type of lymphoma, with a poor

prognosis.⁷ It presents with rapidly growing tumour masses, characteristically on the legs of elderly women. It has been described in association with chronic lymphoedema and malignant melanoma as well as RA.⁸ It may masquerade as a chronic non-healing venous ulcer, making a clinical diagnosis difficult, especially in a patient with an extensive history of severe venous ulceration, as in this case.⁹

This case report is consistent with the observation of an overrepresentation of haematological malignancy in patients with RA. Uniquely interesting is that this patient presented with CML having had no exposure to disease modifying drugs, and had only had 3 months' treatment with busulfan 10 years previously when she presented with lymphoma. Alkylating agents are carcinogenic, particularly leukaemogenic, but reports suggest this is after many years of treatment.¹⁰ We found no cases of lymphoma after busulfan treatment. In this case study it is thus difficult to implicate immunosuppressive drugs in the aetiology of these malignancies, which supports the hypothesis that an increase in haematological malignancy in RA may be a result of the disease itself rather than its treatment.

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None of the authors have any competing interest.

Figure 1 Cutaneous diffuse large B cell lymphoma in a patient with RA who had had little exposure to disease modifying drugs.

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Accepted 2 June 2005

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Altered peptide ligands regulate muscarinic acetylcholine receptor reactive T cells of patients with Sjögren's syndrome

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Ann Rheum Dis 2006;**65**:269–271. doi: 10.1136/ard.2005.039065

In the generation of Sjögren's syndrome (SS), CD4 positive $\alpha\beta$ T cells have a crucial role. Previous studies have provided evidence about the T cell receptor (TCR) V β and V α genes on these T cells, and sequence analysis of the CDR3 region indicates the presence of some conserved amino acid motifs, supporting the notion that infiltrating T cells recognise relatively few epitopes on autoantigens.¹

Candidate autoantigens recognised by T cells that infiltrate the labial salivary glands of patients with SS have been analysed, and Ro/SSA 52 kDa,² α -amylase, heat shock protein, and TCR BV6 have been identified, although Ro/SSA 52 kDa reactive T cells were not increased in peripheral blood.³ Gordon *et al* indicated that anti-M3R autoantibodies occurred in SS and were associated with the sicca symptoms.⁴ Recently, we provided evidence for the presence of autoantibodies against the second extracellular domain of muscarinic acetylcholine receptor (M3R) in a subgroup of patients with SS.⁵ The M3R is an interesting molecule, because this portion has an important role in intracellular signalling,⁵ although the function of anti-M3R autoantibodies remains unknown.

The mechanism through which a peptide is recognised by a TCR is flexible. If the amino acid residue of the peptide ligands for TCR is substituted by a different amino acid and can still bind to major histocompatibility complex molecules (altered peptide ligand), such an altered peptide ligand could regulate the activation of T cells. Several studies have shown that an altered peptide ligand could induce differential cytokine secretion, anergy, and antagonism of the response to the wild-type antigens.^{6,7} The altered peptide ligand has the potential of being used therapeutically against T cell mediated diseases such as autoimmune diseases and allergic disorders.

As an extension to our previous study,⁵ we focused in the present study on M3R reactive T cells and analysed T cell epitopes and their altered peptide ligands with the aim of regulating T cell proliferation and autoantibody production. The 25mer synthetic amino acids encoding the second extracellular domain of M3R (KRTVPPGECFIQLSEPTITF

GTAI, AA213–237) were used as the antigen for T cells, and the number of interferon (IFN) γ producing T cells was counted by flow cytometry using a magnetic activated cell sorting (MACS) secretion assay. The proportion of IFN γ -producing T cells among peripheral blood mononuclear cells (PBMCs) was high in two of five patients with primary SS (pSS) and two of four patients with secondary SS (sSS), compared with the level in four healthy control subjects (HC) (fig 1A). Three patients with SS and M3R reactive T cells (pSS-2, and sSS-1, 2) had the HLA-DR B1*0901 allele and the other patient (pSS-1) had HLA-DR B1*1502 and *0803 alleles. The 25mer amino acids contain the anchored motifs that bind to HLA-DR B1*0901. Thus, IFN γ production by T cells should be due to the recognition of antigen on the HLA molecule by the TCR on T cells.

The results shown in fig 1 were obtained as follows. Blood samples were collected from five Japanese patients with pSS and four Japanese patients with sSS followed up at the University of Tsukuba Hospital. All patients with SS satisfied both the Japanese Ministry of Health criteria for the classification of SS⁸ and the revised EU-US criteria⁹. We also recruited four HC from our university. Approval for this study was granted from the local ethics committee, and written informed consent was obtained from all patients and HC who participated in this study.

Their HLA-DR allele was examined by the SSOP-PCR method, as described elsewhere. A 15mer peptide (VPPGECFIQLSEPT) (M3R AA216–230) corresponding to the sequence of the second extracellular loop domain was also synthesised (Kurabo Industries, Osaka, Japan). PBMCs were purified with Ficoll-Paque and 5×10^6 cells were cocultured with 10 μ g of M3R peptide (25mer) in 1 ml of RPMI-1640 with 10% of human AB serum (Sigma, St Louis, MO) for 12 hours at 37°C. As a positive control, 1 μ g of staphylococcal enterotoxin B (Toxin Technology Inc, USA) was used. IFN γ -producing cells were identified by the MACS cytokine secretion assay (Miltenyi Biotec, Bergisch Gladbach, Germany). Briefly, the cells were incubated with 20 μ g of IFN γ detection antibody (Ab; Miltenyi Biotec), 20 μ g of