Immune response to 70 kD heat shock proteins and rheumatoid arthritis: implication of HLA-DRB1*0401

Sirs,

The amino acid motif QKRAA of HLA-DRB1*0401 carries susceptibility to develop rheumatoid arthritis (RA) (1). How this motif influences the development of RA is unknown. Because the QKRAA motif is adjacent to the peptide-binding pocket of HLA-DR, it could preferentially bind antigens and present them to T cells. We previously reported that the QKRAA motif of HLA-DRB1*0401 is a binding motif for 70 kD heat shock proteins (2-5). These findings raise the question whether immunisation against bacterial hsp70s during bacterial infection can trigger immunisation against self hsp70 in people expressing HLA-DRB1*0401 and whether this has anything to do with the development of RA. To test, if hsp70s can constitute RA triggering antigens after bacterial infection and if the abnormal reactivity is correlated with particular HLA-DR alleles, T cell precursor frequencies to hsp70s were evaluated in peripheral blood of controls, RA patients and patients who received Bacillus Calmette-Guérin (BCG) therapy, a treatment for bladder cancer "mimicking" repeated bacterial infections.

We first evaluated the frequency of T cell precursors against three hsp70s, the bacterial dnaK and the human hsp72 and hsp73 in 7 HLA-DRB1*0401 controls and 14 non HLA-DRB1*0401 controls and performed the same direct limiting dilution analysis in 4 HLA-DRB1*0401 RA patients and in 7 non HLA-DRB1*0401 RA patients (6). Proliferation was considered positive when the T cell precursor number reached 10 cells per million.

We found that the frequency of T cells recognizing hsp70s is not higher in HLA-DRB1*0401 controls than in controls who do not express HLA-DRB1*0401. We observed the same result in RA patients whether they expressed HLA-DRB1*0401 or not. Moreover, we observed no difference in antibody production against hsp70s between HLA-DRB1*0401 and non HLA-DRB1*0401 controls and RA patients. In summary, we found no evidence that HLA-DRB1*0401 can influence T cell responses and antibody production to hsp70s in controls and RA patients (data not shown).

To evaluate if HLA-DRB1*0401 subjects have increased numbers of T cells capable of proliferating to bacterial and human hsp70s after bacterial infection and if this has anything to do with the development of arthritis, we used peripheral blood T cells from patients with bladder carcinoma treated with BCG therapy. Different reasons explained this choice. Adjuvant intravesical BCG therapy is a common procedure after bladder resection to prevent tumour proliferation (7). BCG therapy, started one month after tumour resection, includes 6 weekly intravesical instillations with BCG. In this context, repeated BCG instillations "mimic" repeated bacterial infections in patients. Moreover, although BCG therapy is generally well-tolerated, one of its most severe complications is arthritis (8). The mechanism of action of BCG-induced arthritis is unclear, however the autoimmune response seems to occur in genetically "susceptible" patients. It is therefore possible that HLA-DRB1*0401 may play a role in the presentation of hsp70 in the immune response initiated by BCG instillations. In this context, we evaluated T cell precursor frequencies to hsp70s in the peripheral blood of 10 patients with bladder carcinoma treated with BCG. Five patients underwent event free BCG therapy and 5 patients developed arthritis after BCG instillation. We found that T cell precursor frequencies to human hsp70s is exaggerated in patients receiving 6 BCG instillations but this strong immune response is not associated with any particular HLA-DR allele (Table I). We observed the same results in patients who developed arthritis after intravesical BCG therapy for bladder cancer. In contrast, no increase in antibody response was observed against human hsp70s in BCG therapy patients. In conclusion, T cell responses against human hsp70s are common after BCG therapy and do not seem to be associated with arthritis even in HLA-DRB1*0401 patients.

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Table I. T cell precursor frequencies to heat shock proteins in BCG therapy patients

Patients	HLA-DRB1* type	Instillations	T cell precursor frequencies 10^{-6}						
			E. coli proteins	E. coli DnaK	Mycobacterium HSP65	Mycobacterium HSP71	Human HSP72	Human HSP73	BIP
PG	0401/14	0	0	0	0	0	0	0	2
		3	14	0	0	0	0	5	5
		6	0	10	50	7	20	20	50
DM	03/13	0	0	0	2	2	9	5	1
		3	0	2	10	2	0	2	0
		6	5	7	11	8	50	50	5
OB	0102/7	0	13	8	0	13	4	0	8
		3	0	0	2	13	6	0	0
		6	13	50	50	50	13	13	7
FV	13/15	0	1	7	0	0	5	6	5
		3	2	5	3	0	5	13	7
		6	0	7	0	3	0	0	0
CJB	0102/13	0	0	7	0	0	0	0	5
		3	3	0	0	0	2	4	0
		6	0	0	0	7	0	0	0
Patients with arthritis									
SJ	0401/11	6	0	5	3	10	20	>20	0
SR	03/03	6	8	0	NT	NT	20	25	NT
GJ	11/13	6	0	4	0	0	20	10	0
BJ	1/15	6	14	10	10	10	8	8	10
MT	14/16	6	0	3	0	0	0	0	0

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Beneficial effect of talc pleurodesis in refractory pleural effusion due to Behçet's disease

Sirs,

In patients with Behçet's disease (BD), pleural effusion due to pleural vasculitis or superior vena cava syndrome (SVCS) can be seen in the course of the disease (1, 2). The development of SVCS in BD is mostly related with vascular inflammation and thrombotic tendency (1-4). Treatment options for pleural effusion due to SVCS encompass immunosuppressives and video-assisted thoracic surgery (1-4). To the best of our knowledge, there exists no report in the literature showing the beneficial effect of talc pleurodesis in recurrent pleural effusion due to SVCS of BD.

A 24-year-old man was admitted to our hospital with dyspnoea and swelling in the face and neck in January 2005. He had been diagnosed as having BD associated with Budd-Chiari syndrome (BCS) in 2003. Because of the presence of low-grade oesophageal varicosities and mild impaired hepatic functions, he had been taking warfarin with low intensity and azathioprine 50 mg/day. On physical examination, he was found to have a venous fullness of the neck, hepatosplenomegaly and decreased respiration voice in the right hemi thorax. Laboratory findings were as follows: haemoglobin 10.4 g/dl, WBC 9000 mm^3, erythrocyte sedimentation rate (ESR) 85 mm/h, total protein 5.8 mg/dl, albumin 2.7 mg/dl (N: 0.01-0.3). Chest radiogram showed a pleural effusion in the right hemi thorax. A thorax computed tomography disclosed thrombotic occlusion of the superior vena cava and the brachiocephalic vein. Because of the severity of his dyspnoea, thoracentesis was performed. The white cell count of the pleural fluid was 200/mm^3. The pleural fluid was transudate. Thoracentesis had to be repeated seven times within a period of just 10 days to relieve his respiratory distress due to relapse in accumulation of the pleural fluid. The thoracoscopic imaging showed dilated vessels on both pleura and diaphragm surfaces. Following the pleural biopsy of the area affected, pleurodesis was performed with talc of 5 gr. Pathological evaluation of his biopsy specimen showed a reactive mesothelial hyperplasia characterized by multinuclear mesothelial cells without evidence of vasculitis. The patient has been safe from accumulation of the pleural fluid over the past twelve months with no recurrence.

A number of pulmonary problems associated with BD have been defined, which problems can be classified into three groups (1) pulmonary artery aneurysm (2) pulmonary parenchymal chances, and (3) others including pulmonary artery occlusion, pleural effusion and pulmonary obstructive airway disease (3, 4). Pleural effusion can be transudative, exudative or chylous. It has been reported that the causes of pleural effusion were thrombosis of the superior vena cava or vasculitis of the pleura (3, 4). In our case, no evidence of vasculitis was found in the pleural biopsy. Because our patients also had BCS, pleural effusion may have originated from ascetic fluid. It has been reported that pleural effusion can be seen in the course of hepatocellular failure due to transportation of ascitic fluid into the pleural space (5). Our patient had been having BCS for twenty four months and he had only minimal ascites. Moreover, we ascribe recurrent pleural effusion to superior cava syndrome rather than to minimal ascites. We also rule out the possibility the hypoalbuminemia could have triggered development of the pleural fluid, considering the fact that the patient had already been suffering from hypoalbunemia over the past two years.

The treatment options for pleural effusion seen in BD include immunosuppressives (ISs), together with anticoagulation and video-assisted thoracic surgery. Guralnick *et al*. reported a case with chylothorax due to SVCS as a presenting symptom of BD (6). Chylothorax has been regressed by a combination of ISs and anticoagulation in this case. As to our patient, AZA and warfarin, which may result in hepatotoxicity and haemorrhage, were given in small doses, because he had low-grade oesophageal varicosities and mild impaired hepatic functions. We assumed that pleurodesis could be an alternative approach to our case. Talc pleurodesis has been successfully used in persistent and recurrent pleural effusion due to malign diseases (7). Our case is the first of its kind in that pleurodesis with talc was performed for the treatment of recurrent pleural effusion due to SVCS of BD. In BD, there is an increased inflammatory response to the exogenous stimulating factors. A typical example of this entity is pathergy reaction that may be induced by needle prick and applying urate crystalline to the skin (8). Theoretically, it could be speculated that talc application may result in an inflammatory response in pleural space as a result of the pathergy reaction. However, our case did not develop any inflammatory reaction, nor has he referred to our clinic with recurrent pleural effusion since his discharge. Despite this promising result, we suggest that further clinical experience is needed to be able to consider talc pleurodesis in the treatment of recurrent pleural effusion due to BD.

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