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Risk of chemotherapy-induced pulmonary fibrosis is associated with polymorphic tumour necrosis factor-a2 gene

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Risk of chemotherapy-induced pulmonary fibrosis is associated with polymorphic tumour necrosis factor-a2 gene. J. Libura, F. Bettens, A. Radkowski, J.M. Tiercy, P.F. Piguet. ©ERS Journals Ltd 2002.

ABSTRACT: In some patients, chemotherapy (CHT) of cancer can result in pulmonary inflammation and fibrosis, eventually leading to respiratory insufficiency.

As animal studies have underlined the importance of major histocompatibility complex (MHC) genes in the susceptibility to bleomycin (BLM)-induced pulmonary fibrosis, the authors typed human leukocyte antigen-DR (HLA-DR) and tumor necrosis factor (TNF) genes in patients treated for Hodgkin's disease by a therapy including bleomycin.

Patients were divided into pulmonary responders (PR) (n=21) or nonresponders (PNR) (n=20) on the basis of pulmonary alterations detected on chest radiography and the cumulated amount of BLM injected. The incidence of TNFa2, a microsatellite allele in the promoter region of the TNFB gene reported to be associated with increased TNF-α production, was significantly higher in PR than PNR (65% *versus* 19%). HLA-DRB1*15 showed a weak but nonsignificant association with the PR phenotype (50% *versus* 14%), as well as HLA-DRB1*03 (30% *versus* 19%) and TNFA-308*2 (30% *versus* 14 %). TNFa2 and DR15 were independent risk factors and the occurrence of either genetic marker was 85% *versus* 29% in the PR and PNR groups respectively.

Thus, the polymorphic TNFa2 microsatellite is associated with a risk of chemotherapy-induced pulmonary fibrosis.

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Various drugs are used for the chemotherapy (CHT) of cancer and the effectiveness of this treatment is limited by the susceptibility of the cancer cells and of the normal host tissues. In the latter case, damage to the lung can result in inflammation and fibrosis, sometimes with long delays after the administration of CHT [1]. Bleomycin (BLM), a chemotherapeuthic drug that belongs to a family of complex glycopeptides derived from *Streptomyces verticillis* with potent tumour killing properties, is known to induce a high incidence of pulmonary complications [2]. Toxicity of BLM for cancer cells is believed to be due to its capacity to react with deoxyribonucleic acid (DNA), producing chain scission. In addition, the pulmonary inflammation and fibrosis induced by BLM are aggravated by other interventions, such as oxygen administration or radiotherapy (RT). However, the incidence of pulmonary fibrosis (PF) concerns only a minority of patients treated with BLM, the reason for which is not yet known.

Administration of BLM in rodents is a classical model used to investigate the pathogenesis of PF [2]. In mice it has been reported that susceptibility to BLM-induced fibrosis varies to a great extent between strains. Some strains (*e.g.* CBA, C57BL6) are susceptible and others, notably the Balb/c, are nonresponders [3]. More refined analysis using mice with different major histocompatibility complexes (MHC) has shown that the nonresponder phenotype is linked to the H-2d (D) haplotype and, more precisely, to its telomeric part, which comprises the D and tumour necrosis factor (TNF) loci [4]. In addition, the importance of TNF in the development of PF has been demonstrated by showing that responder, but not nonresponder, mice increase their TNF production in the lung in response to BLM injection and that the severity of the fibrosis can be markedly attenuated by the administration of TNF antagonists [5, 6]. The immune response might also be important in the outcome of BLM-induced PF in mice since the disease is attenuated by T-lymphocyte depletion or in T-lymphocyte deprived mice [2, 5]. Furthermore, T-lymphocyte depletion abrogates the BLM-induced increase in TNF messenger ribonucleic acid in the lung [5]. These observations suggest that the MHC class II genes implicated in T-lymphocyte activation might also play a role in the initiation of the inflammatory cascade.

In humans, the genes encoding TNF-α (TNFA) and the homologous but independently-regulated TNF-β (TNFB) are located within the class III region of the MHC, between human leukocyte antigen (HLA)-B and HLA-DR [7]. The lymphotoxin-β gene (LTB) lies 3 kilobases centrometric to TNFA and codes for a 33 kilodaltons glycoprotein. Lymphotoxin-β forms a complex with TNF-β on the surface of activated T, B and lymphokine-activated killer cells [8]. Numerous reports have documented polymorphisms within the TNF genes, which influence TNF production and disease susceptibility. HLA-DR3/DR4 subjects have been reported to produce higher TNF-α levels [9]. A single nucleotide polymorphism (G to A transition) at position -308 in the promoter region of TNFA has been found to increase TNF-α transcription 6–8-fold [10]. The TNFA-308*2 allele appears to be a risk factor for diseases such as asthma, severe malaria, diabetes, or coeliac disease [11–13]. Polymorphic microsatellites that flank the TNF genes have also proved to be informative markers of TNF-α production [14, 15]. One of these, TNFa, located upstream of TNFB, has been reported to be associated with high (TNFa2) or low (TNFa6, a4) TNF-α production by blood monocytes [14]. TNFa2 has been reported to be a significant risk factor for insulin-dependent diabetes (IDD) or Leishmaniasis [15, 16].

PF is a complex group of diseases of multiple aetiologies. It is characterized by alveolar inflammation, with remodelling of the alveolar septa and an increase in the content of proteins of the extracellular matrix (ECM), notably collagen-1. As reviewed previously [17], studies of HLA phenotypes and some types of PF have not shown strong associations with HLA. The strongest association reported concerns HLA-DR3 and the susceptibility to PF that is linked with systemic sclerosis [18]. Recently, a higher incidence of the TNFA-308*2 gene was reported in coal miners with pneumoconiosis [19]. There is presently no information regarding the possible association between CHT-induced PF and the HLA phenotype.

In the present study, the incidence of the HLA-DRB1, TNFa and TNFA-308 polymorphisms in groups of patients treated with CHT, including BLM for Hodgkin′s lymphoma (HL), were examined. The radiological response of the lung was graded and related to the total dose of BLM received, allowing classification of patients as pulmonary responders (PR) or pulmonary nonresponders (PNR). The authors report a significant association between the TNFa2 microsatellite allele and susceptibility to CHT-induced PF.

Methods

Patients

Sixty-three patients (31 males) with HL were treated at the Institute of Oncology in Cracow, Poland, and were followed for ⩾2.5 yrs, the median follow-up being 5.4 yrs. The disease was identified on the basis of lymph node biopsy and was staged according to the Ann Arbor system. The median age of patients was 40 yrs. Twelve patients were treated with RT alone and were not investigated further. Ten other patients were not included in the study for reasons including the presence of pulmonary abnormalities detected before treatment (1), an insufficient radiological record or a poor compliance with therapy (9). Forty-one patients with disease stage >II, treated with repeated courses of hybrid CHT regimen containing mechlorethamine, vincristin, procarbazine, and prednisone/doxorubicin, BLM, and vinblastin as an induction therapy, as described by CONNORS *et al.* [20], and for whom clinical and radiological data for ⩾2.5 yrs after the beginning of therapy were available, were investigated further for DNA studies. Three patients were treated with CHT alone. In 38 patients, CHT was followed by supradiaphragmatic RT of 39 Gray, in 26 fractions as consolidative treatment. CHT was administered within 8–40 weeks after diagnosis and the total dose of BLM within the CHT cocktail ranged between 15–509 mg. BLM dose intensity ranged between 1.5–10 $mg \cdot week^{-1}$. Blood donors were used as control for the analysis of HLA and TNF polymorphisms.

Score of pulmonary lesions

The evaluation of the pulmonary response to CHT was based on chest radiography performed before and during the follow-up after therapy. The most recent radiographs, obtained >2.5 yrs after the beginning of therapy, were used for the lung score. Radiological abnormalities consistent with fibrosis, such as linear streaking, regional contraction, pleural thickening, tenting of the diaphragm, retraction of the hilum or mediastinum, lung scaring and patchy/discrete or solid consolidations, were recorded. Lesions were graded in accordance with the radiological criteria described in the Subjective, Objective, Medical and Analytical (SOMA) evaluation of injury score of the European Organization for Research and Treatment of Cancer (EORTC) [21], using a 0–4 scale defined as follows: 0: normal lung; 1: discrete unilateral supradiaphragmatic densities; 2: diffuse and patchy densifications; 3: diffuse and patchy consolidations with area of confluence; and 4: diffuse patchy densification, involving scaring and contractions.

Typing of human leukocyte antigen-DRB1 and tumour necrosis factor genes

DNA was extracted from frozen peripheral blood samples using standard proteinase K and phenol-chloroform procedure. HLA-DRB1 typing was performed by polymerase chain reaction sequence-specific oligonucleotide probes (PCR-SSOP) hybridization [22].

TNFa microsatellite polymorphism was determined by using the PCR primers IR2 5′-GCCTCTAGATTTCATCCAGCCACA and flourescein fluorescence-labelled IR4 5′ CCTCTCTCCCCTGCAACACACA [14]. PCR reactions contained 10 pmol of each primer, 0.4 µg DNA in 1.75 mM $MgCl_2$, 10 mM Tris (pH 8.8), 50 mM KCl buffer, 200 µM deoxyribonucleotide triphosphates, 10 $\mu g \cdot mL^{-1}$ bovine serum

albumin (BSA), and 0.5 units Taq polymerase (Invitrogen BV, Leek, the Netherlands). Cycling conditions were as follows: denaturation for 3 min at 94°C, followed by 24 cycles of 30 s at 94°C/30 s at 54°C (decreasing every cycle by 0.2°C)/30 s at 72°C in a Techne Progene termocycler (Applied Biosystems, Rotkreuz, Switzerland). The PCR (1 μL) product was mixed with formamide and 0.4 μL of internal size standard (TAMRA; Applied Biosystems), heat denatured and analysed by electrophoresis on an ABI 310 (PE Biosystem) capillary DNA sequencer using the Genescan program. The sizes of the PCR products were assigned based on TNFa alleles determined from control cell lines of known genotype: QBL (93 base pairs (bp)), AMALA (95 bp), WT51 (99 bp), YAR (111 bp), HHK (113 bp), corresponding to the TNFa1, TNFa2, TNFa4, TNFa10 and TNFa11 alleles, respectively [14].

TNF-308 diallelic polymorphism (guanine (G)/ adenine (A)) was determined as described previously [23]. Briefly, the TNF-308*1 allele (G at position -308) was determined using primer pair C1/C2 and the TNF-308*2 allele (A at position -308) with the primer pair C1/C3. Primers were as follows. C1: 5′-TCTCGGTTTCTTCTCCATCG; C2: 5′-ATAGGTTTTGAGGGGCATGG; and C3: 5′-ATAGGTTTTGAGGGGCATGA. HLA-DRB primers were included in each reaction as internal PCR controls. Each PCR contained 10 pmol of each of the primers C1/C2 or C1/C3, 3 pmols of the HLA-DR primers DRBP1/DRBP2 [22], 200 ng DNA in 1.75 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.8), 50 mM KCl buffer, 200 μM deoxyribonucleotide triphosphates (dNTPs), 10 $\mu g \cdot mL^{-1}$ BSA and 0.5 units of Taq polymerase. The reaction mixture was denatured for 3 min at 94°C, followed by 10 cycles of 30 s at 95°C/45 s at 65°C/40 s at 72°C and 19 cycles of 30 s at 94°C/75 s at 63°C/45 s at 72°C. PCR products were electrophoresed on a ethidium bromide containing 1.5% agarose gel and visualized under ultraviolet light.

Statistical evaluation

Phenotype frequency was calculated from the number of patients or controls possessing the phenotype divided by the total number of subjects in the sample. Gene frequency was calculated using the Arlequin computer package (Arlequin Version 2.0; Genetics and Biometry Lab, Dept of Anthropology, University of Geneva, Geneva, Switzerland). The association of HLA-DR and TNF alleles within groups of patients was tested using the Chi-squared test. p-Values were corrected (Pc) using the Bonferroni correction applied to all significant differences.

Results

Human leukocyte antigen-DRB1 and tumour necrosis factor polymorphisms in Hodgkin's lymphoma

In order to isolate the effects of CHT on the lung from the effects of the basic disease and to discriminate between the effects of TNF polymorphisms and those of other HLA-DR genes, DNA in all patients and controls was typed for the TNFa, TNF-308 and HLA-DRB1 polymorphisms [24]. The HLA-DRB1 phenotypic frequencies in 41 HL patients and 51 healthy controls are shown in table 1. A somewhat higher frequency of HLA-DRB1*15 and HLA-DRB1*11 was detected in HL patients as compared to normal subjects, whereas DRB1*07 and DRB1*13 were less frequent in the HL patients (table 1). These associations, although nonsignificant, were in accordance with those reported in a study on patients with nodular sclerosing HL [25].

With regard to the TNF polymorphic genes, no significant difference in the distribution of the TNFA-308* alleles was observed between healthy controls and HL patients (table 1), in accordance with a previous study of 36 HL patients [26]. The overall frequency distribution of the TNFa microsatellites in controls was also similar to that reported previously in a normal population [27] and was not different from that observed in HL patients (table 1).

Response of the lung to chemotherapy

As shown on figure 1, the cumulative incidence of pulmonary abnormalities of different grades increased up to ~4 yrs after the beginning of CHT

Table 1. – Human leukocyte antigen-DR and tumour necrosis factor (TNF)a in controls and in Hodgkin lymphoma patients

	Controls		Hodgkin		Chi-squared	p-value	Pc
	n	%	n	%			
Subjects	51		41				
DRB1*01	11	21.6	10	24.4	0.1027	0.748	
DRB1*15	11	21.6	13	31.7	1.2116	0.271	
DRB1*16	3	5.9	7	17.0	1.8964	0.168	
DRB1*03	11	21.6	10	24.4	0.1027	0.748	
DRB1*04	15	29.4	8	19.5	1.1879	0.275	
DRB1*11	12	23.5	15	36.6	1.8684	0.171	
DRB1*12	2	3.9	1	2.4	0.0370	0.847	
DRB1*13	13	25.5	4	9.8	37352	0.053	
DRB1*14	3	5.9	2	4.9	0.0632	0.801	
DRB1*07	14	27.5	4	9.8	4.5222	0.033	0.43
DRB1*08	4	7.8	0	0	1.7404	0.187	
DRB1*09	0		0	0			
DRB1*10	0		2	4.9	0.7665	0.381	
TNFa2	17	33.3	17	41.4	0.6447	0.421	
TNFa3	1	1.9	2	4.9	0.0370	0.847	
TNFa4	7	13.7	9	21.9	1.0704	0.300	
TNFa5	4	7.8	8	19.5	2.7286	0.098	
TNFa6	11	21.5	12	29.3	0.7186	0.396	
TNFa7	6	11.7	11	26.8	3.4240	0.064	
TNFa8	1	1.9	2	4.9	0.0370	0.847	
TNFa9	6	11.7	4	9.7	0.0008	0.976	
TNFa10	13	25.5	18	43.9	3.4487	0.063	
TNFa11	11	21.5	9	21.9	0.0019	0.964	
TNFa12	1	1.9	3	7.3	0.5444	0.460	
TNF-308*1	50	98.0	40	97.6	0.3167	0.573	
TNF-308*2	11	21.6	9	21.9	0.0019	0.964	

Pc: Bonferroni correction.

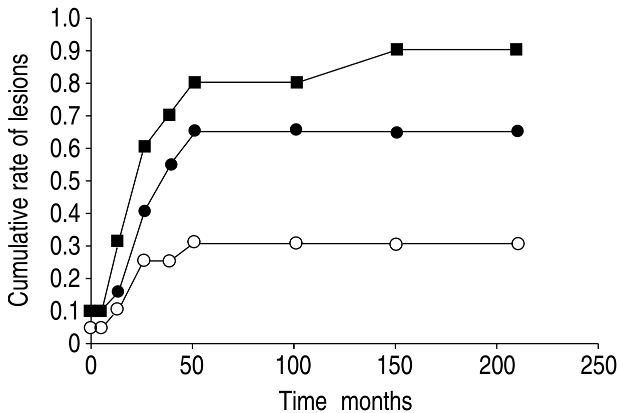

Fig. 1. – Cumulative incidence of pulmonary lesions in patients as a function of time after the initiation of chemotherapy (CHT) (n=3) or CHT associated with radiotherapy (n=38). Lung lesions scored <2, (■), <3 (●) or <4 (○).

and remained relatively stable thereafter. To evaluate responsiveness to CHT, the most recent radiographs, obtained >2.5 yrs after the beginning of CHT, were used. When the lung score was examined in relation to the total dose of BLM injected, no significant correlation was evident (fig. 2).

To evaluate the HLA-DRB1 and TNF genotypes of patients with CHT-induced pulmonary complications, a total of 41 patients treated with CHT alone (n=3) or CHT associated with RT (n=38) for Hodgkin's stage >II were analysed. Twenty-one of these patients had enlarged mediastinal lymph node without radiological involvement of the lung parenchyma. In order to score the response of the lung to CHT, an index of responsiveness, which took into account the lung score determined 4 yrs after the beginning of the CHT, was divided by the total dose of BLM injected in mg (1×10^2), based upon the assumption that the pulmonary response is directly related to the severity of the lung lesions and indirectly related with the total dose of BLM. On the basis of this index, patients were ranked in decreasing order of responsiveness (table 2). Patients with a lung score >2.5 were classified as PR, while those with a score <2.5 were classified as PNR. The mean±SD ages in both groups were 40±12 and 35±15 for PR and PNR, respectively. The male/female ratios were 12/20 and 7/21 in PR and PNR, respectively. Histological types were similar in both groups, with a mixed population of cell types (7 of 15 and 8 of 15 in PR and PNR, respectively). Involvement of the mediastinal LN was present in 11 of 21 of the PNR and 10 of 20 of the PR, *i.e.* it was not associated with the occurrence of parenchymal alterations.

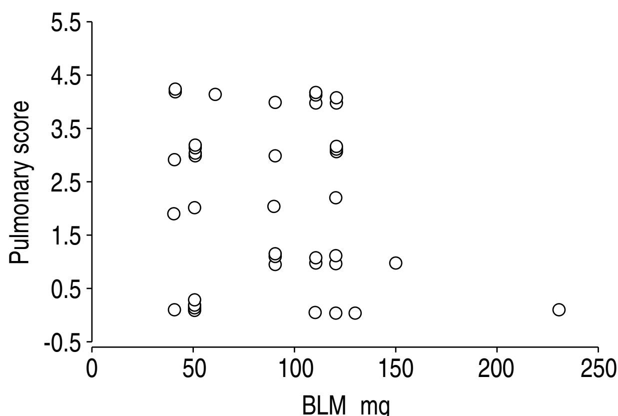

Fig. 2. – Patient pulmonary score in relation to the total dose of bleomycin (BLM) used in chemotherapy. Coeffiecient of determination <0.03.

Human leukocyte antigen-DRB1 and tumour necrosis factor gene polymorphisms in the pulmonary response to chemotherapy

Comparison of the TNFa polymorphisms in the PR and PNR showed a significant increase of TNFa2 in PR (tables 2 and 3). In contrast, the frequency of TNFa2 was similar in normal subjects and in HL patients (table 1). The TNFa6 allele was slightly more frequent and the TNFa4 allele slightly less frequent in PNR individuals. Other polymorphic genes reported to be associated with increased TNF-α production are HLA-DR3 [28] and TNFA-308*2, a diallelic polymorphism located in the promoter of the TNFA gene [9]. These two alleles were increased, although not significantly, in PR patients (table 3). A slight increase of the DRB1*15 genotype in the PR compared to the PNR group (50% *versus* 14%, Pc=0.18) (table 3) was also observed.

A calculation of linkage disequilibrium between the HLA-DRB1 gene and the TNFa loci did not show any significant linkage, neither in the total population of patients and controls (p=0.332) nor in patients (p=0.897) or controls alone (p=0.257). The combined analysis of DRB1*15 and TNFa2 polymorphisms (table 4) showed that a similar percentage of TNFa2-positive patients was found in the DRB1*15-positive (60%) and DRB1*15-negative (70%) PR, whereas this percentage was lower but not markedly different in the DRB1*15-positive (33.3%) and DRB1*15-negative (16.6%) PNR. In controls, the DRB1*15-positive and DRB1*15-negative subjects contained similar proportions of TNFa2, 27.3% and 32.5%, respectively. These figures argue against a linkage between DRB1*15 and TNFa2 and suggest that DRB1*15 and TNFa2 are independent risk factors in BLM-induced fibrosis. In contrast, DRB1*03 and TNF-308*2 exhibited a highly significant linkage disequilibrium (p<0.0001). A linkage of DRB1*03 with TNFa2 has been previously reported and was also evident in the HL patients in this study, whereas DRB1*15 was found to be associated with TNFa11 in the A3-B7-DR2 haplotype [27].

Discussion

The poor or absent correlation between BLM dose and pulmonary response suggests that the genetic constitution of the host is of importance in the occurrence of pulmonary complications, an interpretation

Table 2. – Pulmonary response to chemotherapy (CHT), human leukocyte antigen (HLA) and tumour necrosis factor (TNF) genes

Patient no.#	BLM dose mg		Lung score	Lung responder score¶	HLA-DRB1*	TNF-308	TNFa
	Total	Per week					
1	30	4.2	4	13.3	15/11	1/1	2/11
2+	30	10.0	4	13.3	15/x	1/1	2/6
3	30	8.8	3	10.0	04/11	1/1	2/9
4	60	6.0	4	6.6	03/01	1/2	2/11
5	45	5.2	3	6.6	03/11	1/2	2/2
6	45	5.2	3	6.6	04/11	1/1	6/11
7	45	11.0	3	6.6	01/04	1/1	6/10
8	45	5.2	3	6.6	15/03	1/1	2/9
9	45	10.0	2	4.4	15/01	1/1	6/11
10	90	4.0	4	4.4	15/11	1/1	7/10
11	90	4.5	4	4.4	15/x	1/2	2/11
12	105	2.7	4	3.8	03/13	1/1	2/10
13	110	5.2	4	3.8	16/03	1/2	2/10
14	120	6.0	4	3.3	15/04	1/1	2/10
15	105	1.9	4	3.8	11/x	1/1	4/5
16	30	6.0	1	3.3	11/01	1/1	2/6
17	110	6.0	4	3.3	01/15	1/1	5/9
18	90	9.0	3	3.3	01/15	1/1	6/10
19	105	12.2	3	2.8	03/16	1/2	2/6
20	120	2.8	3	2.5	15/07	1/2	2/6
21+	210	5.0	4	1.9	03/11	1/1	2/4
22	120	15.0	2	1.6	16/01	1/2	4/11
23	90	4.5	1	1.1	07/11	1/1	7/10
24	105	13.0	1	1.0	03/10	1/1	9/11
25	105	5.0	1	1.0	04/13	1/1	2/6
26	90	4.2	1	1.1	03/04	1/1	10/11
27	120	4.0	1	<1	04/07	1/1	7/14
28+	120	4.2	1	<1	13/04	1/1	4/7
29	150	4.3	1	<1	01/x	1/2	5/7
30	135	7.6	0	<1	15/x	1/1	4/10
31	45	6.3	0	<1	16/11	1/1	5/6
32	105	4.1	0	<1	12/13	1/1	11/12
33	90	5.8	0	<1	03/16	2/2	2/4
34	30	2.9	0	<1	11/x	1/1	10/10
35	30	4.0	0	<1	01/11	1/1	6/9
36	45	5.0	0	<1	16/11	1/1	3/10
37	45	5.2	0	<1	11/14	1/1	10/11
38	509	8.7	4	<1	16/11	1/1	10/10
39	90	4.5	0	<1	15/04	1/1	2/8
40	120	5.7	0	<1	15/10	1/1	9/11
41	225	8.0	0	<1	01/07	1/1	4/7

BLM: Bleomycin. #: patients were treated with CHT associated with radiotherapy; ¶: patients were ranked according to the lung responder score; +: patients were treated with CHT alone.

in accordance with the observations made in mice [4]. The present results indicate that TNFa2, a polymorphic TNF allele, is a significant risk factor.

The monocytes from TNFa2 carriers have been reported to produce higher TNF-α levels in response to lipopolysaccharide *in vitro*, although surprisingly this allele did not influence the production of TNF-β [15]. However, in a study of 22 heart transplant patients, no association between the serum TNF-α level and the TNFa2 allele was observed [29]. These discrepancies might be due to the fact that the serum level, which results from a balance between synthesis and clearance, does not reflect synthesis as closely as the culture supernatant from activated leukocytes. Since macrophages are not the exclusive source of TNF during CHT-induced PF [5], the correlation between these polymorphisms and TNF production by other cells remains to be explored. The TNFa2 allele has been reported to be associated with diseases such as IDD [15] and cutaneous Leishmaniasis [16]. DRB1*15 was also weakly associated with risk of pulmonary complication. This finding is intriguing because, so far, this allele has not been reported to be associated with immune or inflammatory diseases. DRB1*15 and TNFa2 were not genetically linked and, accordingly, when the presence of either DRB1*15 or TNFa2 was considered, a highly significant increase in the occurrence of either genetic marker in PR as compared to PNR was evident (85% *versus* 28.6%, Pc=0.0068) (table 3).

In conclusion, the association of the risk of chemotherapy-induced pulmonary complication with

Table 3. – Tumour necrosis factor (TNF)a and human leukocyte antigen (HLA)-DR polymorphic genes in pulmonary responders to chemotherapy

	PR		PNR		Chi-squared	p-value	Pc
	n	%	n	%			
Subject n	20		21				
TNF							
a2	13	65	4	19	8.9122	0.0028	0.033
a3	0	0	1	5	0.0006	0.9803	
a4	1	5	6	29	2.5275	0.1118	
a5	2	10	2	10	0.2257	0.6346	
a6	8	40	3	14	3.4503	0.0632	
a7	1	5	5	24	1.5908	0.2072	
a8	0	0	1	5	0.0006	0.9802	
a9	3	15	3	14	0.1423	0.7059	
a10	6	30	7	33	0.0525	0.8186	
a11	5	25	6	29	0.0665	0.7964	
a12	0	0	1	5	0.0006	0.9802	
a13	0	0	0	0			
TNF-308*2	6	30	3	14	0.7017	0.4022	
HLA-DRB1*03	6	30	4	19	0.2047	0.6509	
HLA-DRB1*15	10	50	3	14	6.0341	0.0140	0.18
HLA-DRB1*15 and/or TNFa2	17	85	6	28.6	13.244	0.0002	0.0068

PR: pulmonary responders; PNR: pulmonary nonresponders; Pc: Bonferroni correction.

Table 4. – Occurrence of tumour necrosis factor (TNF)a2 microsatellite in human leukocyte antigen (HLA)-DRB1*15-positive and DRB1*15-negative subjects

Subject	n	HLA	n	TNFa2 (%)
PR	20	DRB1*15-pos	10	6 (60)
		DRB1*15-neg	10	7 (70)
PNR	21	DRB1*15-pos	3	1 (33.3)
		DRB1*15-neg	18	3 (16.6)
Control	51	DRB1*15-pos	11	3 (27.4)
		DRB1*15-neg	40	13 (32.5)

PR: pulmonary responder; PNR: pulmonary nonresponder.

the polymorphic tumour necrosis factor-a2 microsatellite, reported to increase tumour necrosis factor production, supports a critical role of this cytokine in this disease. In addition, the present results also suggest that the genotyping for human leukocyte antigen-DRB1 and tumour necrosis factor polymorphisms might be useful for the planning of the chemotherapy.

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