Rheumatology 2004;43:423–427 doi:10.1093/rheumatology/keh063
Advance Access publication 16 December 2003

Expression of KIR and C-type lectin receptors in Behçet's disease

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***Objective*. Behçet's disease (BD) is a multisystemic disorder with a possible underlying pathology of immune-mediated vasculitis. Genetic susceptibility associated with HLA-B*51 and B*2702 has been implicated in its pathogenesis. Considering the recently defined regulatory mechanisms of NK cells through HLA class I binding receptors, we hypothesized that interactions of NK and T cells through the NK receptors may be important in the pathogenesis of BD.**

***Methods*. The impact of different expression patterns of HLA-recognizing receptors on NK or T cells was analysed in 51 patients with BD and 32 healthy controls. We used flow cytometry to investigate the expression of KIR3DL1 from the polymorphic killer immunoglobulin-like receptor (KIR) family, which binds a shared HLA-Bw4 motif on HLA-B51 and *2702 alleles, and CD94 from the conserved C-type lectin receptor family, which binds HLA-E. Thirty-three of the BD patients and 19 of the controls carried the same HLA-Bw4 motif.**

***Results*. $CD3^+$ T cells were increased in patients with BD compared with controls (81 *vs* 75%, $P = 0.001$), whereas the NK cells did not show any difference between the two groups. Increased expression of CD94 in BD was observed on $CD16^+CD56^+$ cells (66 *vs* 57, $P = 0.04$) and on $CD3^+$ (7.7 *vs* 4.0, $P < 0.001$) and $CD3^+CD56^+$ (44 *vs* 35, $P = 0.02$) T cells. KIR3DL1 expression on the NK and T cells was not statistically different between the two groups. No effect of HLA-Bw4 motif was observed on the expression of CD94 and KIR3DL1 in both the patients and the controls.**

***Conclusion*. The absence of a correlation between KIR3DL1 expression and HLA-Bw4 motif confirms previous work reporting that the expression of these molecules is regulated separately. Increased expression of CD94 may suggest that NK receptors play a pathogenic or regulatory role in BD.**

KEY WORDS: Behçet's disease, NK cell, KIR3DL1, CD94, HLA-B51, HLA-Bw4.

Behçet's disease (BD) is an inflammatory disorder characterized by recurrent attacks of oral and genital aphthous ulcerations, uveitis and skin lesions [1]. It is now recognized as a systemic disease also affecting the joints, all types and sizes of blood vessels, the lungs and the central nervous and gastrointestinal systems, with vasculitis as the main underlying pathology.

The aetiopathogenesis of BD is unknown. However, immunological abnormalities triggered by some microbial agents or other environmental factors in genetically susceptible individuals are claimed to play an important role. Both innate and adaptive immune systems are activated in BD, and there is a proinflammatory and Th1 type of cytokine profile [2, 3].

Natural killer (NK) cells are lymphocytes of innate immunity that both exert cytotoxic activity and produce cytokines. However, an increased number of NK cells, with lower activity, has been reported in the peripheral blood of patients with active BD [4, 5]. An elevated number of NK cells with a high anti-candidial index in the inactive stage of BD, compared with healthy controls, has also been shown [6, 7]. More recently, increased expression of CD16 or CD56 antigens on peripheral blood $CD4^+$ cells of BD patients without an increase in NK cells has been demonstrated [8]. The proportion of T lymphocytes expressing both T-cell and NK-cell markers, $CD56^+$ T cells (a subgroup of natural killer T cells), has also been found to be higher in BD patients compared with patients with other causes of uveitis [9]. Hence, the data on the involvement of NK cells in the pathogenesis of BD are still controversial.

Recent developments in NK cell research have revealed broad functional diversity of these cells. The clonal diversity of NK cells is due to the combinatorial expression of different cell surface molecules, including several receptors for polymorphic epitopes of MHC class I. The class I specificity of individual NK cells is determined by an array of receptors present at the cell surface [10]. The first group of NK receptors, the killer immunoglobulin-like receptors (KIR), bind to conserved epitopes shared by different allelic groups of HLA class I molecules, and engagement of these receptors has been shown to be associated with selective inhibition or activation of NK- or T-cell-mediated cytotoxicity [10]. A member of this group of receptors, KIR3DL1 (p70), found on NK cells, binds residues 77–83 of HLA-B alleles, which have been designated Bw4 by serology and are shared by the BD-associated HLA-B alleles B*5101 and B*2702 [11]. On the other hand, CD94/NKG2 heterodimers, members of the other complementing group of NK receptors, namely C-type lectin like receptors, bind the non-classical class Ib HLA-E molecules, loaded with the nanomeric peptides of the leader sequences of the other HLA class I molecules. These heterodimeric receptors are inhibitory if CD94 binds with NKG2-A (p43) and activatory when CD94 binds with

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Submitted 27 June 2003; revised version accepted 30 September 2003.

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NKG2-C (p39). The actions of NK cells are mediated by the integration of inhibitory and activating signals sent by these receptors upon ligand binding [12].

The expression of the CD94 receptor on NK as well as T cells varies depending on the inducing environment, whereas the expression of the KIR family, KIR3DL1 in particular, is genetically determined [12, 13]. In this study, we aimed to investigate the expression of two types of NK-cell receptors, HLA-Bw4 binding polymorphic KIR (KIR3DL1) and the conserved CD94 on the peripheral blood cells of patients with BD and healthy controls, and to evaluate their possible effects on HLA-B51-related pathogenic mechanisms in BD.

Materials and methods

Patients and controls

The patient group consisted of 51 patients with BD (29 male, 22 female) followed at the BD Outpatient Clinic of the Division of Rheumatology, Istanbul University. All patients met the International Study Group criteria for the classification of BD [14]. Their mean age was 41.5 ± 9.7 (range 22–67) yr, the mean age at onset was 28.7 ± 8.0 (range 13–43) yr and the mean disease duration was 12.9 ± 8.1 (range 4–41) yr. All of the patients were on treatment (colchicines, $n = 49$; corticosteroids, $n = 25$; azathioprine, $n = 13$; cyclophosphamide, $n = 3$; cyclosporin, $n = 2$) and did not describe a particular BD-related manifestation at the time of blood collection. These patients were typed for full HLA-B alleles by molecular methods (Table 1) [15]. Thirty-seven of 51 BD patients were HLA-Bw4-positive. Cells from a group of 32 ethnically and HLA-B-typed, non-related healthy volunteers were tested as controls. Nineteen of the control group had Bw4-containing alleles. The local ethics committee (Ethics Committee of Istanbul Faculty of Medicine) approved the study and all study subjects provided written informed consent according to the Declaration of Helsinki prior to blood collection.

Monoclonal antibodies

Fluorescence-conjugated monoclonal antibodies (mAb) and their target antigens used in the study were as follows: CD45/FITC (fluorescein isothiocyanate)-CD14/R-PE (R-phycoerythrin) (to distinguish the non-monocyte/macrophage leucocytes) (Ancell, Bayport, MN, USA); CD3-Cy-Chrome (anti-TCR/ε-chain, T-cell marker), CD16-Cy-Chrome (anti-FcγRIII, NK marker), CD56-R-PE (anti-NCAM, NK marker), CD94-FITC (70 kDa dimer = Kp43, NK receptor), KIR3DL1-FITC (anti-NKB1, 70 kDa, KIR) and the corresponding three fluorescence isotype controls (Pharmingen, San Diego, CA, USA).

Flow cytometry

The reactivity of mAb with blood cells was assessed by direct staining of EDTA (ethylenediamine tetraacetate) blood samples with three different fluorescence labels. For every antibody combination, 0.5 μg of antibody per 10^6 cells was incubated on ice for 20 min (10 μl/100 μl blood) and lysis buffer was added (0.15 M NH_4Cl, 0.01 M $KHCO_3$, 100 μM EDTA). After 10 min, the samples were centrifuged twice at 1200 r.p.m. for 10 min to wash away the antibodies. All samples were then tested in a flow cytometer and the results were analysed using CellQuest software (FACSort; Becton Dickinson, Mountain View, CA, USA). Results are expressed as percentages of the respective positive cells in the mononuclear cell gate.

Statistical analysis

The parameters compared between patients and controls were subjected to the non-parametric Mann–Whitney *U*-test.

Results

Cell populations

We analysed fresh peripheral blood leucocytes for CD3, CD16 and CD56 expression in the patients and healthy controls. As shown in the Table 2, the mean value of $CD3^+$ cells was increased in the peripheral blood of BD patients ($P = 0.002$). The total numbers of NK cells ($CD56^+/CD16^+$) and $CD56^+$ T cells did not reveal any difference between BD patients and controls.

Expression of the non-polymorphic NK receptor CD94 was increased in the BD group compared with the healthy controls (14.1 *vs* 9.8%, $P = 0.001$) (Table 2). The increased expression of CD94 was found on $CD16^+CD56^+$ NK cells (65.6 *vs* 56.7%, $P = 0.03$), $CD56^-CD3^+$ (7.7 *vs* 4.0%, $P < 0.001$) and $CD56^+CD3^+$ (43.7 *vs* 34.6%, $P = 0.02$) T cells simultaneously (Fig. 1).

The expression of KIR3DL1 receptor did not differ between the patients and controls (3.4 ± 3.9 and 2.9 ± 2.9% respectively). The individual expression ratios of KIR3DL1 varied extensively among the groups (between 0.0 and 20.4%). This variation was detected on NK cells ($CD56/CD16/KIR3DL1^+$) of both BD patients (16.2 ± 16.2%) and controls (17.5 ± 13.8%) within the ranges of 0–76.2 and 0–48.5% respectively. KIR3DL1 expression on $CD56^+CD3^+$ T cells was also heterogeneous: mean values were 9.0 ± 10.5 and 7.6 ± 9.0% respectively (Fig. 1). Moreover, the expression of KIR3DL1 on $CD56^+CD16^+$ cells correlated with the expression on $CD56^+CD3^+$ cells both in BD patients ($P = 0.01$, $r = 0.36$) and in the control group ($P = 0.005$, $r = 0.49$). According to the recent study by Gardiner *et al.* [13], the binding level of KIR3DL1-specific antibody also revealed different reaction patterns: 55% of BD and 44% of control cells showed low binding of DX9, whereas 6 and 9% respectively showed a high level of binding. Among all tested samples, 12% of the BD cells and 13% of control cells did not bind DX9 at all. However, the distribution with respect to these patterns was also not different between the two groups.

TABLE 1. Phenotype frequencies of HLA-B alleles in patients with BD ($n = 51$) and healthy controls ($n = 32$)

HLA-B allele	Bw4[a]	Bw4-80Ile[b]	BD patients (%)	Healthy controls (%)
B*51	+	+	26 (51)	13 (42)
Others	+	+	6 (12)	6 (19)
	+	−	5 (10)	0 (0)
	−	−	14 (27)	12 (39)

[a]An HLA-B serological epitope defined by the amino acids at positions 77–83.
[b]One of the HLA-Bw4 motifs, with isoleucine at position 80.

TABLE 2. Cell subgroup distribution in BD and control groups (mean percentages)

	BD patients $n = 51$	Controls $n = 32$	*P*
$CD3^+$	81.1 ± 8.5	75.4 ± 6.6	0.001
$CD56^+CD16^+$	10.0 ± 6.0	10.4 ± 6.5	
$CD56^+CD3^+$	8.3 ± 5.7	6.2 ± 8.9	
$KIR3DL1^+$	3.4 ± 3.9	2.9 ± 2.9	
$CD94^+$	14.1 ± 6.3	9.8 ± 4.5	0.001
$CD56^+CD16^+KIR3DL1^+$	16.2 ± 16.2	17.5 ± 13.8	
$CD56^+CD3^+KIR3DL1^+$	9.0 ± 10.5	7.6 ± 9.0	
$CD56^-CD3^+KIR3DL1^+$	1.5 ± 1.8	0.8 ± 0.9	
$CD56^+CD16^+CD94^+$	65.6 ± 16.1	56.7 ± 18.8	0.04
$CD56^+CD3^+CD94^+$	43.7 ± 17.1	34.6 ± 15.9	0.02
$CD56^-CD3^+CD94^+$	7.7 ± 4.5	4.0 ± 2.3	< 0.001

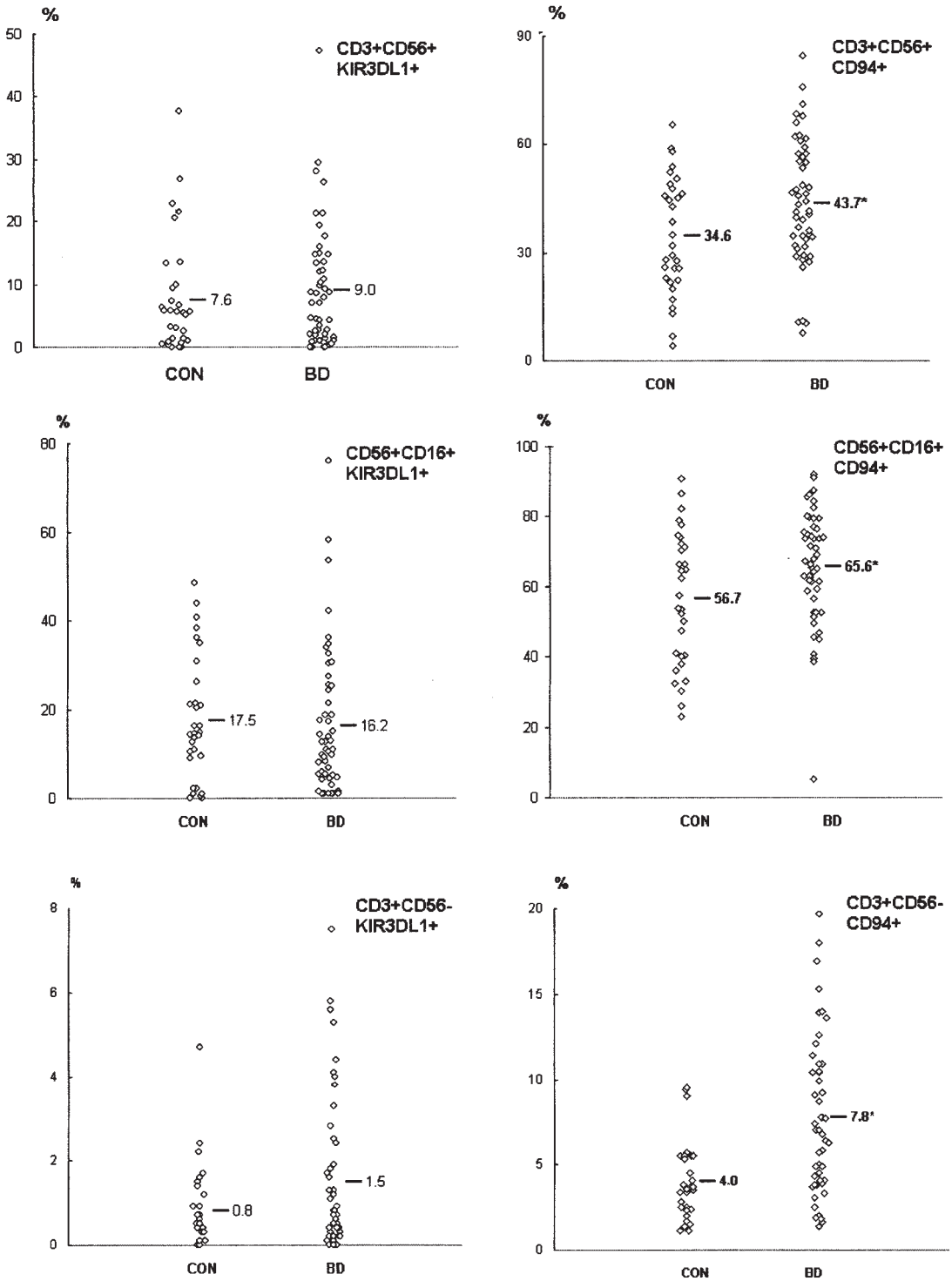

FIG. 1. The distribution of KIR3DL1 and CD94 receptors on NK (CD56+ CD16+) and T cells (CD3+ CD56+/−) of BD patients and controls. Significant differences are marked with an asterisk and are indicated in Table 2.

Receptor expression related to HLA-B distribution

We did a subgroup analysis to investigate whether the BD-associated increase in Bw4-bearing alleles in BD patients influenced the heterogeneous expression of KIR3DL1 and CD94. The expression of KIR3DL1 was compared between the patients and controls, who were classified into subgroups according to the presence or absence of Bw4 antigen, which is found on both the B51 and the B*2702 molecule. Alleles containing the Bw4 sequence [16] were present in 19 out of 32 controls (59.3%) and in 37 out of 51 patients (72.5%) studied for receptor expression; of these $Bw4^+$ alleles, 68% (13/19) and 70% (26/37) were alleles with isoleucine at position 80, respectively (Table 1). A slight increase in $CD3^+CD56^+$ cells in $Bw4^+$ controls was prominent compared with $Bw4^-$ alleles (7.8 *vs* 4%). The HLA-B alleles with the Bw4 sequence did not have any significant influence on the expression of KIR3DL1 or the high or low binding of anti-KIR3DL1 antibody in the patients and controls (Table 3). There was no effect of

TABLE 3. The distribution of subsets in BD and control groups according to the presence or absence of the HLA-Bw4Ile80 motif

	BD patients		Controls	
	Bw4[a] ($n=32$)	Other[b] ($n=19$)	Bw4[a] ($n=19$)	Other[b] ($n=13$)
KIR3DL1	3.4 ± 3	3.6 ± 5	2.8 ± 2	3.0 ± 3
No binding	12.5	10.5	10.5	15.4
Low binding	53.1	57.8	52.6	30.7
Bimodal	31.2	21	31.5	38.5
High binding	3.1	10	5.2	15.4
CD94	12.9 ± 5	15.2 ± 7	9.2 ± 4	10.8 ± 5
$CD16^+CD56^+$	9.6 ± 5	10.3 ± 7	10.2 ± 7	10.7 ± 5
$CD3^+CD56^+$	8.4 ± 6	8.2 ± 5	7.8 ± 11	4.0 ± 2
$CD16^+CD56^+$KIR3DL1	17.5 ± 18	13.9 ± 13	19.8 ± 14	14.4 ± 13
$CD3^+CD56^+$KIR3DL1	7.5 ± 8	11.5 ± 13	9.7 ± 11	4.6 ± 4
$CD16^+CD56^+$CD94	55.4 ± 19	58.5 ± 18	63.3 ± 17	65.9 ± 13
$CD3^+CD56^+$CD94	40.1 ± 17	49.7 ± 16	37.3 ± 16	30.9 ± 15

Data are mean percentage and S.D.

KIR3DL1 expression ratios are grouped according to DX9 binding patterns as low, bimodal, high and no binding within the same groups.

[a]Presence of the HLA-Bw4Ile80 motif.

[b]Absence of the HLA-Bw4Ile80 motif.

the HLA-B alleles or their Bw4 motifs on the increased expression of CD94 on $CD56^+CD16^+$ and $CD3^+CD56^+$ cells (Table 3).

Discussion

The recently described interaction of NK cell receptors with HLA class I molecules on target cells has opened a new perspective on the function of HLA expression and its regulatory influences. In this study, the expression of a member of each NK receptor subgroup, namely KIR3DL1 and CD94, which are expressed mainly on NK cells but also on T cells, was evaluated in patients with BD in order to look for putative functional implications of HLA-B51 association with BD [17–22].

In the peripheral blood, the NK cell population, which we defined as both $CD16^+$ and $CD56^+$, did not differ between BD patients and controls in the present study, which is similar to the findings of some previous reports [8]. The discrepancies between these results and other work [6, 7] could be related to clinical status (different types and activities of BD manifestations) and the number of patients, or to different treatment modalities. The definition of NK cells by the presence of CD56 and absence of CD3 in other studies and the limitations of immunofluorescence methods, due to the number of monoclonals used in the same assay, may also have contributed to these different results.

T cells and NK cells appear to have evolved complementary functions in the immune response, T cells responding to antigen in the context of MHC and NK cells responding to cells that have lost MHC class I expression, according to the 'missing self' hypothesis [23]. A possible regulatory role of NK receptors in the pathogenesis of BD has been supported by the enhanced CD94 expression. Increased expression of CD94 over a relatively wide range was detected on $CD56^+CD16^+$ NK cells and on $CD3^+$ T cells in BD. CD94 is reported to be present on most NK cells, with variable fluorescent staining intensity corresponding to functionally distinct subsets [24]. The expression pattern of CD94 on NK cells has revealed that inhibitory and activating pathways are differently coupled to this receptor depending on the associated molecules, which belong to the NKG2 family and require CD94 for surface expression [25]. The molecules associated with CD94 have been not determined in the present study and the functional relevance of the increase in CD94 expression on NK cells and other T cells, including $\gamma\delta$ T cells, in BD needs to be explored. The expression of molecules associated with CD94 in relation to disease activity, type of organ involvement and treatment will be evaluated further in BD patients.

The CD94 on T cells may be expressed primarily by $CD8^+$ cells of the memory phenotype, as shown by Mingari *et al.* [26, 27] by *in vitro* stimulation with cytokines, superantigens or alloantigens. They reported that the expression of inhibitory NK receptors, such as CD94/NKG2A, can be a regulatory mechanism in the fine tuning of the T-cell receptor-mediated responses of cytotoxic T cells which have acquired NK-like activity, and this may be important in the pathogenesis of BD. The increased serum levels of interleukin (IL)-12 in active and inactive states of BD is consistent with the proposed effect of these cytokines on CD94 expression [28, 29]. The possibility of *in vivo* cytokine-induced (IL-12, IL-15 or transforming growth factor β) expression of CD94 and associated NKG2 molecules [30] on T cells and their functional role in BD also needs to be investigated further.

Recent investigations support a direct pathogenic role for HLA-B51 in the pathogenesis of BD, and there may be various possible ways in which HLA-B51 can interact with T and NK cells. We have recently identified a weak association of HLA-B*2702 with BD [15]. Since HLA-B*2702 and B51 share the same KIR binding sequence, we have hypothesized that HLA-B51 may exert its pathogenic action by binding these receptors on NK and cytotoxic T cells. In the present study, we tested the effects of different distributions of HLA-B alleles in BD on the expression of HLA-recognizing receptors on NK or T cells. Our results did not reveal an effect of increased frequency of the HLA-B51 allele in BD on KIR3DL1 expression. The frequency of $KIR3DL1^+$ cells reacting with DX9 antibody showed a wide range (0–20.4%), and the mean frequency did not differ between patients and controls. NKB1 (DX9) expression on $CD56^+CD3^-$ cells in healthy individuals has been reported as being between 0.1 and 61% (mean, 14%) [31]. More recently, different cell surface phenotypes detected with DX9 antibody have been shown to be due to the expression of different alleles of the KIR3DL1 gene [13]. Although functional differences among these alleles have not yet been elucidated, the variable expression of KIR3DL1 (or reactivity with DX9 antibody) in our samples and the reported 'abnormal' expression in Japanese BD patients [32] may be related to KIR gene polymorphisms. This should be investigated at the allelic level.

The absence of an association between KIR3DL1 expression and the HLA-Bw4 motif confirms previous work showing that the these molecules are subject to separate regulation [16], but does not exclude the possible interaction and involvement of these molecules in the pathogenesis of BD. Evidently, epistatic interaction between the activating *KIR* allele *KIR3DS1* and HLA-B Bw4-80Ile has been demonstrated in progression to AIDS [34]. It has also been shown that the activating and inhibitory KIR receptors and their ligands may interact in a coordinated fashion and that, in the presence of respective HLA ligands, corresponding inhibitory and activating KIRs may neutralize their counteracting effects [35]. In the present work, only the expression level of the inhibitory receptor subtype of the KIR3 family was studied in relation to HLA-B alleles, whereas the activating receptors of the KIR3 and KIR2 families have been found to be associated with AIDS, and with psoriatic arthritis and rheumatoid arthritis, depending on the accompanying HLA-B or HLA-C alleles, respectively [34–36]. The study of activating receptors of KIR3 family members binding HLA-Bw4 alleles is under way.

In conclusion, increased expression of CD94 may suggest that NK cell receptors play a pathogenic or regulatory role in BD. The importance of CD94 expression in BD needs to be explored in functional studies.

Acknowledgements

This study was supported by the Istanbul University Research Fund (Project No. 1076). The work of AG was also supported

by the Turkish Academy of Sciences, in the framework of the Young Scientist Award Program (EA-TUBA-GEBIP/2001-1-1).

Rheumatology	Key messages
	The expression of CD94 is increased in BD. Increased presence of CD94 is demonstrated on NK cells as well as on T cells. KIR3DL1 expression is not different in BD compared with controls.

References

1. Shimizu T, Ehrlich GE, Inaba G, Hayashi K. Behçet disease (Behçet syndrome). Semin Arthritis Rheum 1979;8:223–6.
2. Gul A. Behcet's disease: an update on the pathogenesis. Clin Exp Rheumatol 2001;19(5 Suppl. 24):S6–12.
3. Direskeneli H. Behcet's disease: infectious aetiology, new autoantigens, and HLA-B51. Ann Rheum Dis 2001;60:996–1002.
4. Hamzaoui K, Ayed K, Hamza M, Touraine JL. Natural killer cells in Behcet's disease. Clin Exp Immunol 1988;71:126–31.
5. Kaneko F, Takahashi Y, Muramatsu R *et al*. Natural killer cell numbers and function in peripheral lymphoid cells in Behçet's disease. Br J Dermatol 1985;113:313–8.
6. Onder M, Bozkurt M, Gürer MA, Gülekon A, Sezgin P, Imir T. Natural cellular cytotoxicity in Behçet's disease. J Dermatol 1994;21:239–43.
7. Deniz G, Dum G, Aktas E, Fresko I, Kaygusuz A, Yazici H. Neutrophil functions and anti-candidal activity of CD3-NK cells in Behcet's disease. 14th European Immunology Meeting, 23–27 September 2000, Abstract 434.
8. Eksioglu-Demiralp E, Direskeneli H, Ergun T, Fresko I, Akoglu T. Increased CD4+CD16+ and CD4+CD56+ T cell subsets in Behçet's disease. Rheumatol Int 1999;19:23–6.
9. Yato H, Matsumoto Y. $CD56^+$ T cells in the peripheral blood of uveitis patients. Br J Ophthalmol 1999;83:1386–8.
10. Lanier LL. NK cell receptors. Annu Rev Immunol 1998;16:359–93.
11. Voorter CE, van der Vlies S, Kik M, van den Berg-Loonen EM. Unexpected Bw4 and Bw6 reactivity patterns in new alleles. Tissue Antigens 2000;56:363–70.
12. McQueen KL, Parham P. Variable receptors controlling activation and inhibition of NK cells. Curr Opin Immunol 2002;14:615–21.
13. Gardiner CM, Guethlein LA, Shilling HG *et al*. Different NK cell surface phenotypes defined by the DX9 antibody are due to KIR3DL1 gene polymorphism. J Immunol 2001;166:2992–3001.
14. International Study Group for Behcet's Disease. Criteria for diagnosis of Behçet's disease. Lancet 1990;335:1078–80.
15. Gul A, Uyar FA, Inanc M *et al*. A weak association of HLA-B*2702 with Behcet's disease. Genes Immun 2002;3:368–72.
16. Gumperz JE, Barber LD, Valiante NM *et al*. Conserved and variable residues within the Bw4 motif of HLA-B make separable contributions to recognition by the NKB1 killer cell-inhibitory receptor. J Immunol 1997;158:5237–41.
17. Lehner T, Batchelor JR, Challacombe SJ, Kennedy L. An immunogenetic basis for the tissue involvement in Behçet's syndrome. Immunology 1979;37:895–900.
18. Muftuoglu AU, Yazici H, Yurdakul S *et al*. Behçet's disease: Lack of correlation of clinical manifestations with HLA antigens. Tissue Antigens 1981;17:226–30.
19. Ohno S, Ohguchi M, Hirose S, Matsuda H, Wakisaka A, Aizawa M. Close association of HLA-Bw51 with Behçet's disease. Arch Ophthalmol 1982;100:1445–58.
20. Chajek-Shaul T, Pisanty S, Knobler H *et al*. HLA-B51 may serve as an immunogenetic marker for a subgroup of patients with Behçet's syndrome. Am J Med 1987;83:666–72.
21. Verity DH, Wallace GR, Vaughan RW *et al*. HLA and tumour necrosis factor (TNF) polymorphisms in ocular Behcet's disease. Tissue Antigens 1999;54:264–72.
22. Yazici H, Yurdakul S, Hamuryudan V. Behçet's syndrome. Curr Opin Rheumatol 1999;11:53–7.
23. Ljunggren HG, Karre K. In search of 'missing self': MHC molecules and NK recognition. Immunol Today 1990;11:237–44.
24. Lopez-Botet M, Perez-Villar JJ, Carretero M *et al*. Structure and function of the CD94 C-type lectin receptor complex involved in recognition of HLA class I molecules. Immunol Rev 1997;155:165–74.
25. Perez-Villar JJ, Melero I, Rodriguez A *et al*. Functional ambivalence of the Kp43 (CD94) NK cell-associated surface antigen. J Immunol 1995;154:5779–88.
26. Mingari MC, Ponte M, Bertone S *et al*. HLA class I-specific inhibitory receptors in human T lymphocytes: Interleukin 15-induced expression of CD94/NKG2A in superantigen- or alloantigen-activated CD8+ T cells. Proc Natl Acad Sci USA 1998;95:1172–7.
27. Mingari MC, Ponte M, Vitale C, Bellomo R, Moretta L. Expression of HLA class I-specific inhibitory receptors in human cytolytic T lymphocytes: A regulated mechanism that controls T cell activation and function. Hum Immunol 2000;61:44–50.
28. Turan B, Gallati H, Erdi H, Gurler A, Michel BA, Villiger PM. Systemic levels of the T cell regulatory cytokines IL-10 and IL-12 in Behcet's disease: soluble TNFR-75 as a biological marker of disease activity. J Rheumatol 1997;24:128–32.
29. Frassanito MA, Dammacco R, Cafforio P, Dammacco F. Th1 polarization of the immune response in Behcet's disease: a putative pathogenetic role of interleukin-12. Arthritis Rheum 1999; 42:1967–74.
30. Derre L, Corvaisier M, Pandolfino MC, Diez E, Jotereau F, Gervois N. Expression of CD94/NKG2-A on human T lymphocytes is induced by IL-12: Implications for adoptive immunotherapy. J Immunol 2002;168:4864–70.
31. Litwin V, Gumperz J, Parham P, Phillips JH, Lanier LL. NKB1: A natural killer cell receptor involved in the recognition of polymorphic HLA-B molecules. J Exp Med 1994;180:537–43.
32. Taneko M, Shimoyama Y, Kashiwakura JI, Nagafuchi H, Sakane T, Suzuki N. Abnormal killer inhibitory receptor expression on natural killer cells in patients with Behcet's disease. Rheumatol Int 2003. PubMed ahead.
33. Gumperz JE, Valiante NM, Parham P, Lanier LL, Tyan D. Heterogeneous phenotypes of the expression of the NKB1 natural killer cell class I receptor among individuals of different human histocompatibility leukocyte antigens types appear genetically regulated, but not linked to major histocompatibility complex haplotype. J Exp Med 1996;183:1817–27.
34. Martin PM, Gao X, Lee JH *et al*. Epistatic interaction between *KIR3DS1* and *HLA-B* delays the progression to AIDS. Nat Genet 2002;31:429–34.
35. Martin MP, Nelson G, Lee JH *et al*. Cutting edge: susceptibility to psoriatic arthritis: Influence of activating killer Ig-like receptor genes in the absence of specific HLA-C alleles. J Immunol 2002;169: 2818–22.
36. Yen JH, Moore BE, Nakajima T *et al*. Major histocompatibility complex class I-recognizing receptors are disease risk genes in rheumatoid arthritis. J Exp Med 2001;193:1159–67.