Arthritis Research & Therapy Volume 5 Supplement 2, 2003

Meeting abstracts

1st Workshop of the International Society for Behçet’s Disease (ISBD) on Pathophysiology and Treatment of Behçet’s Disease

Kühtai, Austria, 2–5 April, 2003

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This workshop was supported in part by Merck Austria and the Scientific Society of Hematology, Oncology and Immunology, Innsbruck Austria

Received: 7 July 2003 Published: 9 September 2003

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Introduction

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Arthritis Res Ther 2003 5(Suppl 2):1 (DOI 10.1186/ar976)

For this meeting of two Working Groups of the ISBD, the background to our present knowledge is reviewed, including the following: the original description of Behçet’s disease (BD) by Hippocrates in the 5th century BC and the 20th century description with the adoption of the eponymous title of ‘Behçet’s disease’; the lack of a specific diagnostic test but noting the pathergy test and the association with HLA-B51; description of the multisystem manifestations of the disease and the prevalence of these manifestations, including the differences in different parts of the world; and the pathological classification of BD as a vasculitis.

Nevertheless the diagnosis and classification of the condition depends on the acumen of the physician in diagnosis of the individual patient in the routine clinical situation, and the classification of groups of patients with the disease for inclusion into clinical studies and trials. The older ‘diagnostic schemes’ (e.g. Japanese, Mason and Barnes, O’Duffy, Dilson) should be discarded and the International Classification of BD used as an entry criterion for clinical studies and trials.

The natural history of BD still requires further study with particular reference to being able to predict the type of disease (mucocutaneous, ocular, neuro, etc.), the localization of manifestations, the likelihood of recurrences and their duration, and the overall severity of the disease in the individual patient.

Basic research – pathogenesis (pro-bacterial), immunology (e.g. CD4+CD28− T cells) and gender association (prevalence, severity and response to treatment) – must continue and may proceed more quickly if coordinated on a multicentre, multinational basis.

Therapeutic trials continue but to date there are surprisingly few controlled studies (e.g. colchicine, azathioprine and interferon-α). It is imperative that future trials be properly controlled, and open trials reserved for very preliminary ‘pilot’ studies. Controlled trials (either single or double blind; versus placebo or other comparison drug) are required at present, in particular for the following: IFN-α – optimum dosage regimen with regard to both efficacy and tolerance (side effects); anti-TNF-α, and possibly of antibiotics. Again these may be progressed faster on a coordinated multicentre, multinational basis but with the prior determination of updated entry criteria and outcome measures.

The ISBD, as a medical research society, and through its Working Groups, should be a vehicle for the organization and coordination of studies – single centre, multicentre in a single country, or multicentre on a multinational basis. The Working Groups for Clinical Trials and Treatment and for Basic Research should work towards the coordination of clinical studies and trials with agreed entry criteria – diagnostic classification and disease manifestations and activity; outcome measures; and protocols, including statistical methodology. Multinational coordination through the ISBD may lead to the necessary availability of appropriate funding and secretarial assistance for such trials and studies.

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Perspectives of the Working Group on Drug Trials including Collaborative Trials of the ISBD and rationale for new clinical trials

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Arthritis Res Ther 2003 5(Suppl 2):2 (DOI 10.1186/ar977)

The goals of the working group are to contact and recruit members; to contact other working groups and propagate their work particularly in the context of established criteria of diagnosis and disease activity in Behçet’s disease (BD); to exchange experience via direct contact, home page, journal and/or newsletter; to plan collaborative trials; to contact pharmaceutical companies and research institutes for possible cooperation in clinical trials; to prepare a workshop on clinical trials at ISBD congresses; and to support and alert patients concerning treatment modalities.

Additional clinical trials are needed. From both the physicians’ and the patients’ perspective, current therapy does not satisfy the goals of treatment (e.g. preventing blindness in ocular BD, preventing morbidity and mortality of neurologic and vascular events, improving the quality of life). Serious adverse effects may result from current therapies. Professor S Assaad-Khalil reported the results of a retrospective evaluation of therapeutic agents commonly used in BD, which he had recently carried out in 127 patients (110 males, 17 females; 20–65 years old) randomly selected from the Alexandria BD registry to assess the efficacy of different therapeutic agents in daily practice for a mean duration of 11.07 years by the same group of observers. A special focus was made on the ocular sequelae of the disease, with detailed ocular documentation carried out in 254 eyes (visual acuity, intraocular pressure, state of the lens, presence of uveitis, retinal vessels and optic nerve). Of BD patients 16.6% lost effective vision and another 17.6% of the patients had reduced vision lower than 6/60. Uveitis was found in 37.4%, lens opacity in 44.9%, retinal vessel affection occurred in 23.3% and optic nerve affection in 29.9%. In conclusion, it can be seen that eye sequelae...
are still very devastating in BD, with a delay in diagnosis having a significant deleterious effect on the outcome of the disease. There is still no ideal therapeutic regimen resulting in full remission of BD and preventing its sequelae. At present, combination therapy seems to be the most appropriate approach when considering efficacy and safety. However, there is a great need for a controlled and masked multicentre trial to re-evaluate the efficacy and safety of the different therapeutic modalities on a long term basis.

Improvement should be quantified as objectively and accurately as possible, putting into consideration patient’s quality of life, and measuring all adverse effects. Statistics should depend on strict criteria for statistical significance, avoiding post hoc analysis.

## 3 Cytotoxic drugs in ocular lesions of Behçet's disease

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*Arthritis Res Ther 2003 5(Suppl 2):3 (DOI 10.1186/ar978)*

**Introduction** Cytotoxic drugs are the first line treatment for ophthalmologic manifestations of Behçet's disease (BD) despite the advent of the new biological agents. The latter are to be used in intractable inflammatory attacks as they seem to be efficient in a few case studies. Cytotoxic drugs are affordable, easy to use, effective and safe, even in long-term use. They have to be combined to steroids (0.5 mg prednisolone/kg per day as attack dose, then tapering gradually to the patient’s need). All the cytotoxic drugs used were effective (ACR 1966, APLAR 2000).

As all treatment methods with cytotoxic drugs were efficient and had approximately the same efficiency (percentage of eyes with improved VA), all data were pulled together. The advantage to put the data together was to show the result of treatment in long run in real life. Some patients who were resistant to the given treatment were switched to another treatment and if again ineffective to a third or forth treatment. In pulled data the results before the first treatment were compared with those after the last treatment.

**Materials and methods** Patients who had an active posterior uveitis and/or retinal vasculitis were selected for this study. They were 978 patients. Among them, 277 received more than one treatment. The mean duration of eye lesions was 54 months (SD 42.1), with the maximum duration 271 months. The mean follow-up time was 52 months (SD 40.8) with the maximum of follow up 261 months. Comparison was made by the Student paired t test. VA was calculated on a Snellen chart on a scale of 10 on 10. The Activity Indexes (AI) for different compartment of eyes were calculated according to Ben Ezra. Improvement should be quantified as objectively and accurately as possible, putting into consideration patient’s quality of life, and measuring all adverse effects. Statistics should depend on strict criteria for statistical significance, avoiding post hoc analysis.

**Results** The mean VA of all eyes was 3.8. It improved to 4.7 after the treatment (t 9.54, P < 0.000001). Improved eyes were 62%, 18% were unchanged, and 26% were aggravated. The mean AI of anterior uveitis improved from 2.5 to 0.8 (t 18.595, P < 0.000001). Improved eyes were 62%, 12% were unchanged, and 26% were aggravated. The mean AI of posterior uveitis improved from 2.5 to 1.4 (t 11.661, P < 0.000001). Improved eyes were 62%, 12% were unchanged, and 26% were aggravated. The mean AI of retinal vasculitis improved from 2.5 to 1.4 (t 11.661, P < 0.000001). Improved eyes were 62%, 12% were unchanged, and 26% were aggravated. The percentage of improved eyes remained the same, even in the group of patients where the duration of treatment exceeded 9 years.

**Conclusion** The least improved parameter was the visual acuity, which reflects not only the inflammatory index of the eye, but also the chronicity index (cataract, vitreous organization, hemorrhage, vessel necrosis of retina, neovascularization, and optic nerve atrophy). Seventy percent of the eyes improved or maintained their VA, which is quite remarkable for this disease.

## 4 The use of interferon-alfa in Behçet's disease: review of the literature

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*Arthritis Res Ther 2003 5(Suppl 2):4 (DOI 10.1186/ar979)*

**Objective** To evaluate the efficacy and safety of interferon-alfa for the treatment of Behçet’s disease and discuss its possible mechanisms of action.

**Methods** Reports published until July 2002 in all languages were identified by the PubMed database and the Behçet’s disease conference proceedings and abstract booklets. The indexing items used were Behçet and interferon.

**Results** Thirty-two original reports and four selected abstracts were included in the analysis. Systemic IFN-alfa was administered to 405 patients. Two hundred and sixteen patients with acute ocular disease were treated with IFN-alfa. Two hundred and ninety eight patients received IFN-alfa2a and 141 IFN-alfa2b. 85.6% of the patients with mucocutaneous symptoms, 95.8% with arthritis and 95.6% with uveitis exhibited a partial or complete response. Higher IFN doses were more effective than low dose regimens and led up to 56% long term remissions after discontinuation of IFN-alfa. IFN-alfa2a apparently was superior to IFN-alfa2b with more complete remissions, but this probably was due to a bias caused by the larger number of patients treated with IFN-alfa2a. Side effects were dose dependent and similar to those occurring in patients with hepatitis C.

**Conclusions** Although the comparability of the studies is hampered due to different study designs, it can be concluded that IFN-alfa is effective for the treatment of BD. It was effective even in resistant posterior uveitis, where long-term remissions with preservation of visual acuity could be achieved. In contrast, for mucocutaneous symptoms, only partial remissions were reported.

## 5 Efficacy of recombinant human interferon-alpha2a on ocular and extra-ocular manifestations of Behçet’s disease and influence on cells of the immune system: results of an open four center trial

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*Arthritis Res Ther 2003 5(Suppl 2):5 (DOI 10.1186/ar980)*

**Background** Behçet’s disease (BD) is a multisystem vasculitis of unknown origin. Standard treatment comprises systemic immunosuppressive agents. In a study primarily designed for refractory ocular disease we additionally evaluated the efficacy of recombinant human interferon-alpha2a (rhIFN-alpha2a) for the extracocular manifestations.

**Method** Fifty patients were included in the study. rhIFN-alpha2a was applied at a dose of 6 million units subcutaneously daily. Dose

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reduction was performed according to a decision tree until discontinuation. Disease activity was evaluated by the Behçet’s disease activity scoring system and the uveitis scoring system. In parallel, peripheral blood mononuclear cells (PBMC) from 14 patients and 10 healthy controls were isolated, stained with four different fluorescent dyes and measured with a fluorescence activated cell sorter (FACS). Statistical analysis was performed by ANCOVA and Welsh test.

**Results** Response rate of the ocular manifestations was 92%. Visual acuity rose significantly from 0.56 to 0.84 at week 24 ($P < 0.0001$). Posterior uveitis score of the affected eyes fell by 46% in 1 week ($P < 0.001$). Mean BD activity score fell in a dose-dependent fashion by 1.2 points in the first week ($P < 0.0001$) and from 5.8 to 3.3 at week 24. After a mean observation period of 36.4 months, 17 patients are off treatment and disease free for 29.5 months (mean). In the other patients maintenance dosage is 3 million units 3 times weekly. Whereas extraocular manifestations such as genital ulcerations, arthritis, and skin lesions remitted under IFN, this was the case only for 36% of oral aphthous ulcers. The lymphocyte subpopulations showed a significant increase of $\gamma\delta$ positive T cells and NK cells in the patients before treatment when compared with healthy controls. Under IFN treatment, they decreased significantly and almost reached the level of the control group. Additionally, monocytes and B cells increased.

**Conclusion** rhIFN-alpha2a is effective in ocular BD, resulting in significant improvement in vision and complete remission of ocular vasculitis in the majority of the patients. It is also effective for the extraocular manifestations of the disease, although less so for oral aphthous ulcers. A participation of $\gamma\delta$ positive T cells and NK cells in the pathogenesis of BD is implicated; their decrease may explain the mechanism by which IFN-alpha exerts its therapeutic effects, whereas the increase of monocytes and B cells may be responsible for side effects of IFN such as flu-like syndrome and autoimmune phenomena.

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**TNF and anti-TNF agents in Behçet’s disease**

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*Arthritis Res Ther* 2003 5(Suppl 2):6 (DOI 10.1186/ar981)

The rapid response and effectiveness of anti-TNF agents in the treatment of many immune mediated inflammatory disorders draws comparison to the response of rheumatoid arthritis to cortisone, first witnessed over 50 years ago by Philip Hench and colleagues at the Mayo Clinic. It is now known that many of the anti-inflammatory effects of corticosteroids are due to their inhibition of TNF production. A new dimension of efficacy is possible with TNF agents, however, in that inhibition of disease progression is now possible. Might these agents be considered the corticosteroids of the new millennium? Like rheumatoid arthritis and Crohn’s disease, Behçet’s disease is believed to be associated with a Th1-mediated immune response. Increased levels of TNF-$\alpha$ are found in Behçet’s disease and has provided support for the empiric use of anti-TNF-$\alpha$ therapies used in a number of published cases and small case series. Hassard (2001) reported rapid and dramatic improvement in gastrointestinal and extraintestinal symptoms and findings of the disorder after treatment with infliximab. Similar response was seen in two other patients treated by Travis (2001). This experience was followed by the report of Robertson (2001) of a patient free of oral and genital ulcerations for the first time in 10 years after three infusions of infliximab. Remission of mucocutaneous symptoms for one year followed two infusions of infliximab in a patient previously uncontrolled by multiple immunosuppressive agents (Gooossens, 2001). Mucocutaneous lesions remitted with infliximab in a patient with Behçet’s disease associated with rheumatoid arthritis (Rozenbaum, 2002). At EULAR 2002, Turkish investigators reported the results of the first double-masked, placebo-controlled study ($n = 40$) of anti-TNF therapy with etanercept in mucocutaneous Behçet’s disease (Melikoglu, 2002). This agent suppressed disease manifestations with resurgence after the drug was discontinued. Additional recent reports of anti-TNF therapies in Behçet’s disease were presented at several international meetings.

The experience with anti-TNF-$\alpha$ treatments for the ocular manifestations of Behçet’s disease has been growing and very positive. Sfikakis (2001) reported the benefits of infliximab in five patients with panuveitis in Behçet’s disease. This included two patients treated with infliximab therapy without an increase in conventional treatment. At the ACR meeting in 2002 these Greek investigators reported successful monotherapy with infliximab in acute ocular inflammation in Behçet’s disease in 14 patients. The authors suggested that the dramatic and rapid response in these patients would favor the use of this agent over conventional therapy. Positive responses to anti-TNF agents in ocular Behçet’s disease have been documented in numerous case reports in the literature or presentations at international meetings. Significant differences exist between currently available anti-TNF-$\alpha$ agents, including mechanism of TNF-$\alpha$ inhibition, avidity, half life, immunogenicity, ability to bind lymphotxin (TNF-$\beta$), ability to bind membrane bound TNF-$\alpha$, as well as the mode and frequency of administration. One or more of these differences may account for the variable efficacy of these agents in certain diseases, such as Crohn’s disease, and the variable side-effect profile of these drugs. We believe that it is appropriate to study all available agents, including adalimumab, for their efficacy and safety in Behçet’s disease. The efficacy of these new biologic agents for the more serious manifestations of Behçet’s disease, in particular, should be investigated.

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**A review on disease activity scores in Behçet’s disease (BD)**

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*Arthritis Res Ther* 2003 5(Suppl 2):7 (DOI 10.1186/ar982)

Currently there are no laboratory markers that correlate well with the clinical findings in BD. Therefore assessment of disease activity has to be based on history of clinical features. There have been several attempts to develop disease activity measurements: (1) the scheme in use by H Yazici (1984; evaluation depends on features found on the day the patient attends clinic); (2) the Iranian Behçet’s disease Dynamic Measure (IBODAM; F Davatchi 1991; evaluation depends on accurate history of symptoms up to 12 months prior to the date of assessment); and (3) the European scheme (1991; initially developed in the UK by Chamberlain-Barnes-Silman incorporating features of the scheme used by H Yazici). In all three forms scoring of disease activity is based on clinical features only.

In 1994 a workshop was held in Leeds (UK) to arrive at a consensus view about the contents of the activity form with emphasis on the need for clarity and consistency for potential use by clinicians worldwide. The Behçet’s Disease Current Activity Form (BDCAF) was developed, which depends on accurate history of clinical features present during the month prior to the date of assessment. Clinical features in BD vary considerably over time; in order to document this variation, new clinical features present over the preceding 28 days are scored. This represents a compromise between assessing disease activity based on clinical features on day of assessment, which may be unrepresentative of overall disease activity and clinical features present over a longer time period, as in
the IBDDAM, which reduces reliability in terms of accurate recall of symptoms by patients. In BDCAF disease activity rating for oral and genital ulceration, and skin lesions relies solely on the duration of symptoms and does not take into account the size or number of lesions present (which might also reflect activity).

A study from Bhakta BB et al. (Rheumatology 1999) shows that the BDCAF is easy to complete and is a reliable method of assessing and documenting clinical activity in BD patients for use in routine clinical practice, that it has a good interobserver reliability for general disease activity and that there is difficulty in reliably scoring of uncommon manifestations such as large vessel involvement, GI inflammation and nervous system involvement.

A Turkish study (Hamuryudan et al., Rheumatology 1999) to examine interobserver and intraobserver reliability of the Turkish version of BDCAF shows a good intra- and interobserver agreement for orogenital ulcers and eye involvement, a poor agreement between and within observers for their overall impression and individual low kappa scores for erythema nodosum, vascular, CNS and gastrointestinal involvement. A local disease activity index (Lee ES et al., Book of Abstracts, 10th International Congress on Behçet’s disease, Berlin, 2002) was developed by Koreans that attempted to overcome cultural differences. It also excludes any terms that could be biased, such as fatigue or headache.

For the future we propose a setting of a general clinical score, an organ specific clinical score, laboratory assessments and imaging techniques (like MR for CNS involvement, CT/MR-angiogram, FDG-PET for vasculitis, etc.), which enables us to distinguish between chronic or degenerative changes and active lesions whenever possible. Disease related overall damage would be the total of the cumulative old damage and the damage by active disease.

8 Behçet’s disease: from innate to adaptive immunity
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Arthritis Res Ther 2003 5(Suppl 2):8 (DOI 10.1186/ar983)

Both innate and adaptive immune systems are activated in Behçet’s disease (BD) with a proinflammatory and Th1 type cytokine profile. BD might be linked to a specific, primary immune abnormality with a genetic mutation effecting an adhesion molecule, a proinflammatory cytokine/chemokine or a transcription or regulatory factor, which predisposes to early or more intense neutrophil and T cell responses. Increased neutrophil responses to urate crystals and IMLP or superantigen-driven interferon-γ response of T cells suggest a model with these characteristics, which also explains the ‘pathergy’ or ‘skin urate’ tests. MEFV gene mutations, suggested to be specific for familial Mediterranean fever, which decrease the expression of an anti-inflammatory protein ‘pyrine’ from neutrophils, are also described in BD from Turkey.

However, adaptive immune system is also crucial in BD with possibly both external (streptococcal, HSV) and internal antigens driving the pathogenic tissue T cell infiltrations. Heat shock proteins 60 and 70 can also activate innate immune system directly with Toll-like receptors 2 and 4 and provide both an early innate activation and prolonged T cell response. The diverse manifestations of BD responding to different therapeutic agents also suggest the role of organ-specific antigens (retinal-S antigen in uveitis) or genetic predispositions (factor V Leiden in thrombosis) in different BD clinical subsets.

Better characterization of pathogenic immune cell subsets, systemic and local antigens, and abnormal cell activation mechanisms may help to develop more specific and less toxic immunotherapeutic approaches to still unsatisfactorily treated BD in the future.

Acknowledgement The studies by the author and his collaborators are supported with grants from Turkish Scientific and Technical Council (TUBITAK) and Marmara University Research Funds.

9 Migration of dendritic cells into the lymphatics: the Langerhans cell example
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Arthritis Res Ther 2003 5(Suppl 2):9 (DOI 10.1186/ar984)

Dendritic cells, including Langerhans cells of the epidermis and the mucous membranes, are key leukocytes for the initiation of adaptive immune responses as well as for the maintenance of peripheral tolerance. In the former regard they may well play an important, but as yet unrecognized role in the pathogenesis of Behçet’s disease. Epidermal Langerhans cells may serve as a paradigm for their counterparts in the mucosal: These cells efficiently take up (microbial) antigens, they process them into immunogenic MHC–peptide complexes, and they transport this form of antigen to the lymph nodes via lymphatic vessels. Depending on the milieu where Langerhans cells have encountered antigen (inflammatory versus non-inflammatory/steady-state), they make T cells proliferate and acquire effector functions (immunity) or render them unresponsive or even delete them (tolerance), respectively. In addition, plasmacytoid dendritic cells, a recently characterized type of dendritic cells, may also directly trigger innate responses (e.g. by secretion of type I interferons in response to virus). Studying the pathways and the regulation of dendritic cell migration might help to unravel a possible involvement of dendritic cells in Behçet’s disease.

We present observations on the physical obstacles that dendritic cells migrating in the skin have to overcome until they reach dermal lymphatic vessels. Furthermore, we show that migration is critically dependent on the function of matrix metalloproteinases, in particular MMP-2 and MMP-9. It becomes evident that Langerhans cells indeed carry antigens (including self antigens such as melanosomes or apoptoic bodies or tumor antigens such as particular cytokeratins) through the lymphatics. Given these observations it may be worth studying Langerhans cells and dermal dendritic cells in Behçet’s disease.

10 Viral infection of retinal pigment epithelium: a possible role for initiation of Behçet’s disease
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Arthritis Res Ther 2003 5(Suppl 2):10 (DOI 10.1186/ar985)

The in vitro susceptibility of human retinal pigment epithelial cells (RPE) to representative members of different groups of human pathogenic viruses was investigated in this study. Subacute viral infection is known to change the phenotype of infected cells, thereby causing immune-mediated tissue damage. Downregulation of cell surface antigens provides a means of long-term survival of viruses and persistent infection. Therefore the aim of this study was to investigate the capacity of in vitro infection with viruses and the expression of different cell surface molecules on human RPE cells following viral infection with special emphasis on those having immune regulatory functions. Primary cultures of RPE cells were infected with various viruses. We found infection with different
neurotropic viruses, respiratory viruses and enteroviruses whereas no infection was observed with lymphotropic viruses. Cytomegalovirus (CMV) downregulated MHC class I antigens on RPE, whereas coxsackie virus (CVB) and HSV did not alter MHC class I antigen expression. No induction of class II antigens was observed in RPE cells infected with CVB, HSV or CMV. Adhesion molecule ICAM-1 (CD54) was slightly increased after virus infection and the other cell surface molecules did not alter.

Several common human viruses could infect RPE cells. As even animal viruses, such as pseudorabies virus, could infect these cells, it might be possible that transient infections with animal viruses could act as a trigger for ‘autoimmune’ retinal diseases – and under certain circumstances like genetic predisposition or immunologic disorders this could lead to Behçet’s disease in the eye.

11 Regulation of inflammatory CD28- T-helper cells by HLA class I molecules: a new cellular model for Behçet’s disease?
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Arthritis Res Ther 2003 5(Suppl 2):11 (DOI 10.1186/ar986)

From immunogenetical studies we learnt that Behçet’s disease (BD) is associated with HLA-B*51, and to a lesser extent with HLA-B*2702. There may even be a role of MICA genes in the pathogenesis of BD. Various of these MHC class I molecules can be recognized by NK receptors on NK and NK-T-cells independently from peptides. These NK cell receptors may be activating (DS) or inhibitory (DL). Interestingly, increased percentages of CD4+CD16+ and CD4+CD56+ T-cell subsets have already been described in BD patients.

In patients with rheumatoid arthritis and ankylosing spondylitis, unusual proinflammatory and cytotoxic CD4+ T cells marked by the lack of the costimulatory molecule CD28 express stimulatory NK cell receptors on their surface. In rheumatoid arthritis, MHC class I recognizing NK receptors are even considered as disease risk genes. In CD4+CD28- T cells from patients with ankylosing spondylitis we recently showed functional NK cell features and an enrichment of these cells in the CD4+CD25+ T-cell compartment by costimulation with HLA-B27 transfected cells.

We hypothesize that CD4+CD28- T-cells as markers of a chronic inflammatory process are also elevated in BD patients, express MHC class I recognizing NK receptors, and thus recognize HLA-B*51 via NK receptors. This mechanism could explain the chronicity of BD as an MHC class I associated disease.

12 Immunosuppressive effects of gemcitabine in the HSV-induced Behçet’s disease like mouse model
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Arthritis Res Ther 2003 5(Suppl 2):12 (DOI 10.1186/ar987)

Objective To study effects and side effects of gemcitabine (2′,2′-difluorodeoxycytidine, dFdC), a pyrimidine synthesis inhibitor, on skin lesions of a herpes simplex virus-induced Behçet’s disease (BD)-like mouse model.

Methods Dose-escalation studies with dFdC were performed in ICR mice with intraperitoneal application over 5 days. After inoculation of ICR mice with herpes simplex virus and classification as having BD according to a revised Japanese classification, 18 BD-mice were randomly assigned to placebo, 0.06 or 0.12 µg of dFdC/day over 5 days. Serum levels of interleukin (IL)-4, IL-6, IL-10, tumor necrosis factor-α and interferon-γ were determined using ELISA assays.

Results After application of 3 µg dFdC over 5 days, alanine aminotransferase increased (P = 0.032) but all other kidney and liver parameters were unchanged. In BD mice, 5 days of dFdC treatment with 0.06 or 0.12 µg of dFdC/day resulted in a dose-dependent improvement in cutaneous manifestations by more than 60% (P = 0.017). There was no significant change in cytokine levels and none of the cytokine levels correlated with response to treatment.

Conclusion DFdC shows promising effects to improve cutaneous lesions in the herpes simplex virus-induced BD-like mouse model. In this animal model, effects of dFdC on the cytokine profile remained inconclusive.

13 Streptococcal antigen in the pathogenesis of Behçet’s disease
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Arthritis Res Ther 2003 5(Suppl 2):13 (DOI 10.1186/ar988)

Patients with Behçet’s disease (BD) are highly associated with HLA-B51 immunogenetically and tend to be involved with chronic infectious foci, such as tonsillitis and dental caries, by nonpathogenic streptococci in the oral cavity. BD patients were suggested to be hypersensitive to streptococci and we immunohistologically demonstrated the deposits of streptococcal related antigen at infiltrated cells which were adhering to the vascular walls in erythema nodosum (EN)-like lesions. The Japanese Research Group for BD also demonstrated that BD patients showed greater hypersensitivity against streptococcal antigens than normal healthy controls and that the BD symptoms were frequently induced by the skin tests using these antigens and the treatment of the dental caries. Streptococcus sanguis was dominantly isolated from the infectious foci and the strain strongly adhered to the epithelial cells of the oral membrane, which might be correlated with chemotactic activity of neutrophils in the BD lesions. An attempt at cloning and sequencing the bes-1 gene of S. sanguis isolated from BD patients was made and it has been found that the amino acid sequence of the bes-1 gene has more than 60% of homology with the human intraocular peptide brn-3b, which is a POU domain expressed in the retinal ganglion cells. On the other hand, heat shock protein-65 kDa (HSP-65) derived from microbial organisms which had homology with human HSP-60 was shown to be cross-reactive to the serotype of S. sanguis found in BD patients. Recently we recognized the antibody cross-reactivity against human HSP-60 peptide (336-351), which might stimulate T-lymphocytes of BD patients. In order to explain more precisely the relationship between S. sanguis and BD symptoms, we attempted to find bes-1 gene in the various lesions and to detect the antibodies against both bes-1 synthetic peptides and recombinant HSP-60/65 of S. sanguis in sera of BD patients.

Methodology We performed PCR and PCR in situ hybridization (PCR-ISH) on the samples of BD lesions obtained by punch
biopsy and controls using nested primers, which amplify the S. *sanguis* genomic region coding for *bes-1*, including the *brn-3b* homologous site. We also evaluated the antibody responses against *bes-1* peptides and recombinant HSP-60/65.

**Results** We detected the presence of *bes-1* DNA in the samples of EN-like eruptions, and oral and genital aphthous lesions by PCR analysis. PCR-ISH also revealed *bes-1* DNA gene located in the nuclei of the cells adhering to the vessel walls and macrophages infiltrated in EN-like lesions, whereas the antibodies against both *bes-1* peptides and recombinant HSP-60/65 protein have not been detected in sera of these patients.

**Conclusions** The presence of *bes-1* DNA in macrophages infiltrated in the various lesions of BD patients suggests that the infectious foci by *S. sanguis* in the oral cavity are deeply correlated with the various lesions in BD patients. It is speculated that the clinical symptoms appear by the internalization of *bes-1* DNA to macrophages infiltrated, as an extrinsic factor, in BD patients who are associated with HLA-B51-related gene as an intrinsic factor. However, it is not clear how *S. sanguis* infection is correlated with HLA-B51-related gene as the genetic background in BD patients.