Interleukin-17 in Behçet's disease: relation with clinical picture and disease activity

Dina S. Al-Zifzaf[a], Abir N. Mokbel[c], Doaa M. Abdelaziz[b]

Departments of [a]Physical Medicine, Rheumatology, and Rehabilitation, [b]Clinical Pathology, Faculty of Medicine, Ain Shams University, [c]Department of Physical Medicine, Rheumatology, and Rehabilitation, Faculty of Medicine, Cairo University, Cairo, Egypt

Correspondence to Dina S. Al-Zifzaf, MD, Department of Physical Medicine, Rheumatology, and Rehabilitation, Faculty of Medicine, Ain Shams University, Abbasiya, Cairo 11566, Egypt
Tel: +20 100 520 0934; fax: 0226830729; e-mail: drdyassin@gmail.com

Aim
The aim of this study was to assess serum levels of interleukin-17 (IL-17) in patients with Behçet's disease (BD) and to evaluate its relation to the clinical picture and disease activity.

Patients and methods
This case–control study was carried out on 38 patients with BD. A total of 20 age-matched and sex-matched healthy volunteers serving as the control group were also enrolled in this study. All patients were subjected to full history taking, thorough clinical examination, and clinical activity assessment using Behçet's Disease Current Activity Form. Serum IL-17 levels were measured with enzyme-linked immunosorbent assay.

Results
Serum IL-17 levels were significantly elevated among BD patients. Patients with ocular involvement had higher IL-17 levels compared with those without; however, the difference did not reach statistical significance. Patients with neurological involvement had significantly higher IL-17 levels in their serum compared with those without neurological involvement and controls. Twenty-seven patients had active disease, whereas 11 patients had inactive disease. Serum IL-17 levels were significantly elevated in active BD patients. Patients with and without activity showed highly significant rise in IL-17 levels compared with healthy controls. There was no significant correlation between the amount of corticosteroids or colchicine being used and serum IL-17 levels.

Conclusion
IL-17 is elevated among BD patients and further increased with activity, ocular affection, and neuroinvolvement. Our findings suggest that IL-17 exerts an important role in the pathogenesis of BD, thus providing a promising target for novel therapy.

Keywords:
Behçet's disease, interleukin-17, neuro-Behçet, ocular-Behçet

Introduction

Behçet's disease (BD) is a systemic inflammatory disorder. A complex genetic background coupled with innate and adaptive immune system activation causes the diverse clinical manifestations that characterize the clinical picture [1].

Previous studies have suggested that BD is predominated by a Th1-type immune response, as increased Th1-associated cytokines such as interleukin (IL)-12 and tumor necrosis factor (TNF)-α have been documented in BD patients. However, treatment with anti-TNF could only partially prevent the progression of BD, which suggests that other cells seem to be involved in the pathogenesis of BD [2]. Th17 cells were linked to the pathogenesis of various human autoimmune and inflammatory diseases, and IL-17 levels are increased in rheumatoid arthritis, psoriasis, multiple sclerosis, and inflammatory bowel disease [3]. Studies in mice have reported that strategies aimed at suppressing the Th17 cells were proposed as a major pathogenic subset in patients with BD, as these cells proliferate later and

stay in inflammatory tissues longer compared with Th1 cells; they are suggested to be involved in both early neutrophil chemotaxis to tissues and organ-specific autoimmunity [4]. In addition, studies in mice have reported that strategies aimed at suppressing the Th17 cell response were an effective therapeutic approach in experimental autoimmune uveitis [5].

IL-17 (IL-17A) has been described as Th17 cell-derived cytokine and is highly expressed in autoimmune disorders and inflammatory diseases [6]. IL-17 promotes inflammation by inducing various proinflammatory cytokines and chemokines, recruiting neutrophils, enhancing antibody production, and activating T cells [7]. IL-17 has also been known to play a role in inducing the activation of macrophages, fibroblasts, and endothelial cells, and in stimulating these cells to release various mediators such as IL-1, IL-6, IL-8, TNF-α, and granulocyte colony stimulating factor [8].

The mechanisms by which IL-17 induces the expression of proinflammatory mediators may be cell type-

dependent and appear to involve gene transcription and possibly modulation of mRNA processing [9]. IL-17A can be dysregulated in some individuals and thereby contribute to the pathogenesis and/or maintenance of autoimmune and immune-inflammatory disorders [10]. IL-17 probably participates in the inflammatory process of BD, although few studies at hand approached this area [11,12]. Studies of this pathway may warrant its role and provide a novel strategy for the treatment of this disease, especially as biotherapies targeting this cytokine directly or indirectly are currently being developed.

Aim

The aim of this study was to assess serum levels of IL-17 in patients with BD and to evaluate its relation with clinical picture and disease activity.

Patients and methods

This case–control study was carried out on 38 patients with BD fulfilling the international study group criteria for the diagnosis of BD [13]. Patients were recruited from inpatient departments and outpatient clinics of Rheumatology, Rehabilitation, and Physical Medicine of Cairo and Ain Shams University hospitals. Patients with other inflammatory or autoimmune diseases were excluded from the study. A total of 20 age-matched and sex-matched healthy volunteers serving as control group were enrolled in this study. Written informed consents were obtained from all patients and healthy controls. Our study was designed to adhere to the tenets of the declaration of Helsinki.

All patients were subjected to full history taking and thorough clinical examination, with stress on dermatological, musculoskeletal, vascular, neurological, and ophthalmological examination. The skin pathergy test was also performed [14]. Clinical activity was assessed at the time of venipuncture using Behçet's Disease Current Activity Form (BDCAF) [15]. Patients were defined as having active disease by fulfilling at least two of the following criteria within the preceding 4 weeks: oral ulcer, genital ulcer, skin lesions, ocular lesions, arthritis, or urogenital, vascular, gastrointestinal, and neurological system involvements; they were defined as having inactive disease if they had none or one criterion only within 4 weeks [16].

Laboratory investigations included complete blood count using Coulter counter (Coulter Microdiff 18, Fullerton, California, USA), semiquantitative measurement of C-reactive protein with latex agglutination assay (Teco Diagnostics, Anaheim, California, USA), and erythrocyte sedimentation rate (ESR) assessed using the Westergren method.

Serum interleukin-17 assay

A volume of 3 ml venous blood samples was collected in sterile plane tubes and was allowed to stand for 30 min at room temperature and then centrifuged for 5 min. Sera were immediately separated and stored at -70°C until the time of analysis.

Serum IL-17 levels were measured with precoated WKEA Human IL-17 ELISA Kit (WKEA Med Supplies Corp. Inc., Changchun, Jilin, China) in accordance with the manufacturer's instructions. Standard curve was generated using serially diluted standards. Serum IL-17 concentration was calculated using the generated standard curve. The minimum detectable level of the ELISA kit was 10 pg/ml and the kit had no cross-reaction with other human cytokines.

Statistical methods

IBM SPSS statistics (v. 22.0, 2013; IBM Corp., Armonk, New York, USA) was used for data analysis. Data were expressed as median and percentiles for quantitative nonparametric measures. Comparison between two independent groups for nonparametric data was made using the Wilcoxon rank-sum test. The rank Spearman correlation test was performed to study the possible association between two variables in each group for nonparametric data. The probability of error at 0.05 was considered significant, whereas at 0.01 and 0.001 it was considered highly significant.

Results

A total of 38 BD patients were enrolled in this study; of them, 33 (86.8%) were male and five (13.1%) were female patients. Their mean age was 36.08 ± 10.07 years, and their mean disease duration was 8.9 ± 5.98 years. The ESR of the patients ranged from 5 to 83 mm/h, with a mean of 21.55 ± 14.2 mm/h. C-reactive protein was positive in 23 (60.53%) patients and negative in 15 (39.47%) patients. Twenty-seven (71.05%) patients were on corticosteroids (5–30 mg/day) and 10 patients used colchicine (1–1.5 mg/day) as well as corticosteroids for the disease (27.03%). Azathioprine (50–150 mg/day) was being used by 24 (63.16%) patients, and 11 (28.95%) were taking oral anticoagulants (warfarin). Patients' characteristics are represented in Table 1.

Serum IL-17 levels were significantly elevated among BD patients (median 165 pg/ml; 25th percentile:

100 pg/ml; 75th percentile: 257.5 pg/ml) compared with healthy controls (median 75 pg/ml; 25th percentile: 50 pg/ml; 75th percentile: 125 pg/ml; $Z = -4.224$; $P<0.01$) (Fig. 1).

The mean BDCAF of our patients was 2.9 ± 1.42. Twenty-seven patients had active disease, whereas 11 patients had inactive disease. Serum IL-17 levels were significantly elevated in active BD patients (median 200 pg/ml; 25th percentile: 147.5 pg/ml; 75th percentile: 337.5 pg/ml) compared with inactive BD patients (median 75 pg/ml; 25th percentile: 90 pg/ml; 75th percentile: 200 pg/ml; $Z = -1.99$; $P<0.05$). Patients with and without activity showed highly significant rise in IL-17 levels ($P<0.01$) when compared with healthy controls (Fig. 2).

Ocular manifestations were found in 27 patients (some patients had more than one manifestation at the same time), unilateral in eight, and bilateral in 19. Eight had anterior uveitis, 21 had posterior uveitis, and 10 had retinal vasculitis. Patients with ocular involvement had higher IL-17 levels compared with patients without; however, the difference did not reach statistical significance (median 175 pg/ml; 25th percentile: 130 pg/ml; 75th percentile: 281.25 pg/ml and median 140 pg/ml; 25th percentile: 90 pg/ml; 75th percentile: 240 pg/ml, respectively; $P > 0.05$).

Neurological involvement was found in 17 patients (some patients had more than one manifestation at the same time), stroke in seven patients, convulsions in two, behavioral changes in one, ataxia in one, cranial nerve palsy in one, encephalitis in six, and chronic headache in five. Patients with neurological involvement had significantly higher IL-17 levels in their serum when compared with patients without neurological involvement and controls (Fig. 3).

The rank Spearman correlation test revealed the absence of a significant correlation between IL-17 and age of patients, disease duration, ESR or BDCAF score ($P > 0.05$). There was no significant correlation

**Table 1 Clinical manifestations of patients with Behçet's disease**

| Clinical characteristics	Patients ($n = 38$) [n (%)]
Oral ulcers (recurrent)	38 (100)
Genital ulcers (recurrent)	34 (89.5)
Eye involvement	27 (71)
Skin involvement	13 (34.2)
Pathergy test positivity	24 (63.2)
Fatigue	8 (21.1)
Deep venous thrombosis	14 (36.8)
Joint involvement	14 (36.8)
Neurological involvement	17 (44.7)
Hearing impairment	2 (5.3)
Pulmonary involvement	4 (10.5)

Figure 1
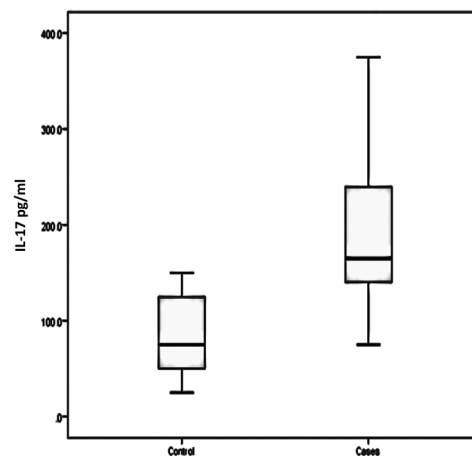
IL-17 levels in serum of Behçet's disease patients compared with controls. IL-17, interleukin-17.

Figure 2
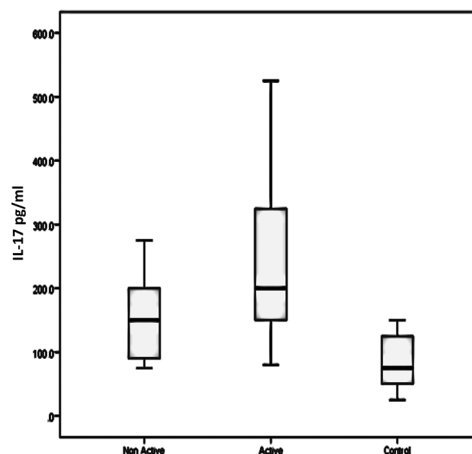
IL-17 levels in serum of patients with active Behçet's disease and inactive disease compared with controls. IL-17, interleukin-17.

Figure 3
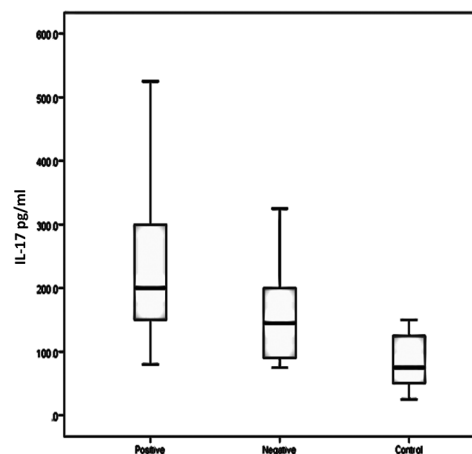
IL-17 levels in serum of BD patients with neurological involvement and BD patients without neurological involvement compared with controls. BD, Behçet's disease; IL-17, interleukin-17.

between the amount of corticosteroids or colchicine being used and the serum IL-17 levels.

Discussion

Behcet's disease (BD) is a multisystem relapsing inflammatory disorder of unknown etiology. An interlacing cytokine network plays an essential role in the evolution and organ damage of this systemic vasculitis of elusive etiopathogenesis [12,17].

Traditionally, BD was regarded as a Th1-mediated inflammatory disease, but prospective observational studies recommend that Th17 cells play a fundamental role in the pathogenesis of BD. IL-17, which is secreted from Th17 cells, activates neutrophils. Hyperactivity of the neutrophils is an important aspect of the immunological abnormalities in BD. Neutrophil hyperactivity and elevated inflammatory cytokine levels are hallmarks of BD. Neutrophils produce inflammatory cytokines, which promote neutrophil activity. This makes a cycle in which elevated and activated neutrophils produce more cytokines, and the latter enhance neutrophil activity [4,18–20]. In addition, several reports have shown that IL-17 cytokine induced the production of various proinflammatory cytokines and chemokines, cell adhesion molecules, and growth factors through a wide range of cell types such as T cells, vascular endothelial cells, dendritic cells, synovial cells, and bronchial epithelial cells [21,22].

Our results showed highly significant elevation in serum IL-17 levels among BD patients compared with healthy controls. These results are consistent with those reported by others [9,23]. Corroborating these results, Takeuchi *et al.* [24] found that stimulated IL-17 production by peripheral blood mononuclear cells was higher in BD patients than in healthy controls. Using intracellular cytokine staining, Shimizu *et al.* [25] found that CD4+ T cells producing IL-17 were increased significantly in BD patients. Other researchers reported high Th17/Th1 ratio in BD patients compared with healthy controls [26]. Moreover, mice that were deficient in IRF-4-binding protein, a protein that inhibits IL-17A production, rapidly developed a large-vessel vasculitis, sharing similarities with human BD because of inappropriate synthesis of IL-17A [27]. Jang *et al.* [28] also identified IL-23R and IL-17F gene polymorphisms, which were associated with susceptibility to BD. Contrary to our findings, one group reported undetectable levels of IL-17 in the sera of BD patients and controls [2].

Our results confirm significantly elevated serum IL-17 levels in active BD patients compared with inactive

BD and healthy controls. Hamzaoui *et al.* [23] reported similar findings; yet, the same group in 2011 observed increased serum IL-17 levels in active BD patients compared with inactive BD patients, whereas no differences in IL-17 levels were found between healthy controls and remission BD patients [9].

Evidence reported by Geri *et al.* [29] support our findings, as they describe a significant and dose-dependent production of IL-17A induced upon the addition of serum from patients with active BD in a sorted CD4+ T cell culture of healthy donors. Wang and colleagues also found an increased frequency of IL-17-producing CD4+ T cells and of IL-17 production by naive T cells cultured under Th17 polarizing conditions in active BD patients. These results suggest that a large amount of IL-17-producing CD4+ T cells and increased IL-17 expression as observed in active BD patients may play a role in the appearance of active inflammation in these patients [30]. In 2013, Na *et al.* [31] confirmed the significantly upregulated IL-17 levels in the serum of active BD patients compared with healthy controls; they found significantly higher frequencies of IL-17 and interferon-γ expressing CD4+ T cells in active BD patients that decreased when the BD disease activity was stabilized. Other groups found an increase in Th17 cells and decrease in T-regulatory cells in the peripheral blood of active BD patients [32].

The rank Spearman correlation test revealed the absence of significant correlation between IL-17 and ESR and BDCAF score in our study. In disagreement with these findings, a significant positive correlation was observed between plasma IL-17A levels and ESR by Hamzaoui *et al.* [9]. This could be because the patients in our study had a narrow range of activity that did not allow the relation to be statistically apparent.

Patients with neurological involvement had significantly higher IL-17 levels in their serum compared with those without neurological involvement and controls. These findings corroborate the findings of Geri *et al.* [29], who demonstrated the presence of IL-17A-producing T cells within the cerebrospinal fluid, brain parenchyma inflammatory infiltrates, and intracerebral blood vessels from three patients with central nervous system involvement in BD.

Our results revealed that patients with ocular involvement had higher IL-17 levels compared with those without; however, the difference did not reach statistical significance. These findings are similar to those reported by Chi *et al.* [2], who showed that IL-17 production by polyclonally stimulated peripheral blood mononuclear cells and activated T cells was markedly elevated in BD patients with active uveitis

compared with that in patients without active uveitis and in controls. Later the same group reported that IL23 and IL-17A were elevated, and the phenomena were observed not only in peripheral blood but also in aqueous fluid in eyes, indicating the given cytokines' implication in pathogenesis of uveitis. After therapeutic intervention of cyclosporin A, IL-17 levels went down significantly. Thus, treatment with cyclosporin A could inhibit the production of both cytokines in association with an amelioration of intraocular inflammation [31].

Conclusion

IL-17 is elevated among BD patients and further increased with activity, ocular affection, and neuroinvolvement. Our findings suggest that IL-17 exerts an important role in the pathogenesis of BD, thus providing a promising target for novel therapy.

References

1 Mat MC, Sevim A, Fresko I, Tüzün Y. Behçet's disease as a systemic disease. Clin Dermatol 2014; **32**:435–442.

2 Chi W, Zhu X, Yang P, Liu X, Lin X, Zhou H, et al. Upregulated IL-23 and IL-17 in Behçet patients with active uveitis. Invest Ophthalmol Vis Sci 2008; **49**:3058–3064.

3 Truchetet ME, Mossalayi MD, Boniface K. IL-17 in the rheumatologist's line of sight. Biomed Res Int 2013; **2013**:295132.

4 Direskeneli H, Fujita H, Akdis CA. Regulation of TH17 and regulatory T cells in patients with Behçet disease. J Allergy Clin Immunol 2011; **128**:665–666.

5 Oh K, Byoun OJ, Ham DI, Kim YS, Lee DS. Invariant NKT cells regulate experimental autoimmune uveitis through inhibition of Th17 differentiation. Eur J Immunol 2011; **41**:392–402.

6 Nakae S, Nambu A, Sudo K, Iwakura Y. Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. J Immunol 2003; **171**:6173–6177.

7 Iwakura Y, Nakae S, Saijo S, Ishigame H. The roles of IL-17A in inflammatory immune responses and host defense against pathogens. Immunol Rev 2008; **226**:57–79.

8 Witowski J, Ksiazek K, Jorres A. Interleukin-17: a mediator of inflammatory responses. Cell Mol Life Sci 2004; **61**:567–579.

9 Hamzaoui K, Borhani Haghighi A, Ghorbel IB, Houman H. RORC and Foxp3 axis in cerebrospinal fluid of patients with neuro-Behcet's disease. J Neuroimmunol 2011; **233**:249–253.

10 Van den Berg WB, McInnes IB. Th17 cells and IL-17A Focus on immunopathogenesis and immunotherapeutics. Semin Arthritis Rheum 2013; **43**:158–170.

11 Leng RX, Chen GM, Pan HF, Ye DQ. The role of IL-23/IL-17 axis in the etiopathogenesis of Behçet's disease. Clin Rheumatol 2010; **29**:1209.

12 Zhou ZY, Chen SL, Shen N, Lu Y. Cytokines and Behcet's disease. Autoimmun Rev 2012; **11**:699–704.

13 International Study Group for Behçet's disease. Criteria for diagnosis of Behcet's disease. Lancet 1990; **335**:1078–1080.

14 Altaç M, Tüzün Y, Yurdakul S, Binyildiz P, Yazici H. The validity of the pathergy test (non-specific skin hyperreactivity) in Behçet's disease: a double-blind study by independent observers. Acta Derm Venereol 1982; **62**:158–159.

15 Bhakta BB, Brennan P, James TE, Chamberlain MA, Noble BA, Silman AJ. Behçet's disease: evaluation of a new instrument to measure clinical activity. Rheumatology 1999; **38**:728–733.

16 Han EC, Cho SB, Ahn KJ, Oh SH, Kim J, Kim DS, et al. Expression of pro-inflammatory protein S100A12 (EN-RAGE) in Behçet's disease and its association with disease activity: a pilot study. Ann Dermatol 2011; **23**:313–320.

17 Peño IC, de las Heras Revilla V, Carbonell BP, di Capua Sacoto D, Ferrer ME, García-Cobos R, González RA. Neurobehcet disease: clinical and demographic characteristics. Eur J Neurol 2012; **19**:1224–1227.

18 Sugita S, Kawazoe Y, Imai A, Kawaguchi T, Horie S, Keino H, et al. Role of IL-22 and TNF-α producing Th22 cells in uveitis patients with Behçet's disease. J Immunol 2013; **190**:5799–5808.

19 Zare Shahneh F, Mohammadian M, Babaloo Z, Baradaran B. New approaches in immunotherapy of Behçet disease. Adv Pharm Bull 2013; **3**:9–11.

20 Neves FS, Spiller F. Possible mechanisms of neutrophil activation in Behçet's disease. Int Immunopharmacol 2013; **17**:1206–1210.

21 Moseley TA, Haudenschild DR, Rose L, Reddi AH. Interleukin-17 family and IL-17 receptors. Cytokine Growth Factor Rev 2003; **14**:155–174.

22 Chi W, Yang P, Zhu X, Wang Y, Chen L, Huang X, Liu X. Production of interleukin-17 in Behçet's disease is inhibited by cyclosporin A. Mol Vis 2010; **19**:880–886.

23 Hamzaoui K, Hamzaoui A, Guemira F, Bessioud M, Hamza M, Ayed K. Cytokine profile in Behçet's disease patients. Relationship with disease activity. Scand J Rheumatol 2002; **31**:205–210.

24 Takeuchi, M, Y Usui, Y Okunuki, L Zhang, J Ma, N Yamakawa, et al. Immune responses to interphotoreceptor retinoid-binding protein and S-antigen in Behçet's patients with uveitis. Invest Ophthalmol Vis Sci 2010; **51**:3067–3075.

25 Shimizu J, Takai K, Fujiwara N, Arimitsu N, Ueda Y, Wakisaka S, et al. Excessive CD4+ T cells co-expressing interleukin-17 and interferon-γ in patients with Behçet's disease. Clin Exp Immunol 2012; **168**:68–74.

26 Kim J, Park JA, Lee EY, Lee YJ, Song YW, Lee EB. Imbalance of Th17 to Th1 cells in Behçet's disease. Clin Exp Rheumatol 2010; **28**:S16–S19.

27 Chen Q, Yang W, Gupta S, Biswas P, Smith P, Bhagat G, Pernis AB. IRF-4-binding protein inhibits interleukin-17 and interleukin-21 production by controlling the activity of IRF-4 transcription factor. Immunity 2008; **29**:899–911.

28 Jang WC, Nam YH, Ahn YC, Lee SH, Park SH, Choe JY, et al. Interleukin-17F gene polymorphisms in Korean patients with Behçet's disease. Rheumatol Int 2008; **29**:173–178.

29 Geri G, Terrier B, Rosenzwajg M, Wechsler B, Touzot M, Seilhean D, et al. Critical role of IL-21 in modulating TH17 and regulatory T cells in Behçet disease. J Allergy Clin Immunol 2011; **128**:655–664.

30 Wang C, Tian Y, Ye Z, Kijlstra A, Zhou Y, Yang P. Decreased interleukin-27 expression is associated with active uveitis in Behçet's disease. Arthritis Res Ther 2014; **16**:R117.

31 Na SY, Park MJ, Park S, Lee ES. Up-regulation of Th17 and related cytokines in Behçet's disease corresponding to disease activity. Clin Exp Rheumatol 2013; **31**:32–40.

32 Pineton de Chambrun M, Wechsler B, Geri G, Cacoub P, Saadoun D. New insights into the pathogenesis of Behçet's disease. Autoimmun Rev 2012; **11**:687–698.