Contribution of Reduced Interleukin-10 Levels to the Pathogenesis of Osteomyelitis in Children with Sickle Cell Disease

Sameh Sarray, Wassim Y. Almawi
College of Medicine & Medical Sciences, Arabian Gulf University, Manama, Bahrain

Osteomyelitis is a significant complication of sickle cell disease (SCD), and several factors contribute to its pathogenesis, including altered expression of proinflammatory and anti-inflammatory cytokines. In view of the role of interleukin-10 (IL-10) as an anti-inflammatory cytokine, we tested the notion that SCD osteomyelitis is associated with a reduction in IL-10 secretion and, hence, precipitation of a proinflammatory state. Study subjects comprised 52 SCD patients with confirmed diagnosis of osteomyelitis and 165 age- and gender-matched SCD patients with negative histories of osteomyelitis. Results obtained showed that IL-10 serum levels in SCD osteomyelitis patients were significantly lower than those of control SCD patients. Receiver operating characteristic (ROC) analysis demonstrated that altered IL-10 serum levels predicted the development of osteomyelitis, and the mean area under ROC curves of IL-10 was 0.810 among SCD patients with osteomyelitis. A systematic shift in IL-10 serum levels toward lower values was seen in osteomyelitis cases, with an increased osteomyelitis risk associated with decreased IL-10 levels. Multivariate logistic regression analyses confirmed the independent association of reduced IL-10 with osteomyelitis after controlling for sickle hemoglobin (HbS), fetal hemoglobin (HbF), platelet count, and white blood cell (WBC) count. These data support the strong association of decreased IL-10 levels with osteomyelitis, thereby supporting a role for IL-10 in osteomyelitis follow-up.

Sickle cell disease (SCD) is a monogenic, inherited anemia with variable clinical manifestations and associated variable disease severity (1), since some patients present with severe complications, while others do not present with any SCD complications (2). Besides vasoocclusive crisis (VOC), osteomyelitis is a common complication of SCD and is a major cause for hospitalization of SCD patients (3). Osteomyelitis can present as an acute or chronic inflammatory destruction of the bone and is characterized by progressive destruction of infected bone and recruitment of osteocytes to the infection sites. These sites include femur, humerus, vertebra, ribs, and sternum (4, 5), although any bone may be infected (3, 5, 6). Osteomyelitis is multifactorial in nature and is influenced by local factors relating to bone lesion and type of infectious microorganism (5, 7), together with an inherited predisposition and immunological dysfunction (3, 8, 9).

Distinguishing the acute presentation of osteomyelitis from VOC relies on clinical assessment (fever and pain on admission, swelling of affected limb, and painful sites) and radiological findings (ultrasound scans, magnetic resonance imaging [MRI]), in combination with elevated C-reactive protein (CRP) and white blood cell (WBC) counts (5, 10). Distinct cytokine profiles were reported during early (1 to 4 months) and late (5 to 12 months) osteomyelitis episodes; the early-episode profiles are highlighted by an increased frequency of high tumor necrosis factor alpha (TNF-α) and interleukin-4 (IL-4) producers, while the late-episode profiles are exemplified by increased frequencies of IL-10, IL-6, and IL-2 producers (11). The expression of proinflammatory cytokines (IL-1, IL-6, TNF-α) was reportedly upregulated in osteomyelitis patients, suggesting an imbalance between proinflammatory and anti-inflammatory mechanisms in the pathogenesis of posttraumatic (12) and tuberculosis (13) osteomyelitis.

Functionally, inflammatory cytokines induce osteomyelitis-associated regulation of osteoclast activity and bone destruction directly (14–17) and/or indirectly through stimulation of the release of key bone-remodeling factors (18). On the other hand, the anti-inflammatory cytokine IL-4, acting by downregulating IL-1 and Th1-type cytokine production (19, 20), was described to be involved in the restoration of damaged bone tissue in osteomyelitis (21). Insofar as the related anti-inflammatory cytokine IL-10 acts as a suppressor of infection-stimulated bone resorption in vivo (22) and was associated with SCD complications (23), the aim of this study was to assess the association between IL-10 serum levels and osteomyelitis in children with SCD.

MATERIALS AND METHODS

Patients and controls. From September 2010 to June 2012, 217 SCD patients (132 males and 85 females) with a mean (± standard deviation [SD]) age of 12.9 ± 8.9 years (range, 1 to 17 years), diagnosed according to hemoglobin (Hb) profile (HBA, sickle Hb [HbS], HbA2, and fetal Hb [HbF]), were enrolled. Patients were assigned to one of two groups—osteomyelitis (n = 52) and no-osteomyelitis SCD patient controls (n = 165)—according to their clinical presentation and laboratory findings.

Diagnosis of osteomyelitis was based on clinical signs, radiological evidence, and laboratory evaluation. Clinical assessment of osteomyelitis included swelling, warmth, tenderness, and inability to bear weight. Radiological findings, with plain radiography or MRI, were used to rule out other causes. In addition, a confirmatory diagnosis of osteomyelitis was based on hematological investigations, in particular the measurement of erythrocyte sedimentation rate (ESR) and CRP levels. While these lacked...
specificity, normal ESR and CRP levels ruled out osteomyelitis. Blood cultures and bone or joint pus aspiration (24) were also used in diagnosing osteomyelitis; in general, a positive blood culture obviated the need for biopsy specimens. The most common infectious organisms in osteomyelitis patients were Salmonella species (n = 51; 78.8%) and Staphylococcus aureus (n = 11; 19.2%). The tibia (n = 38; 64.4%), humerus (n = 14; 23.7%), and femur (n = 7; 11.9%) were the main sites of infection. Treatment of osteomyelitis consisted of intravenous penicillin and a cephalosporin, used either alone or in combination. The control group included afibrile SCD patients with no history of SCD-associated bone involvement, and these patients were matched with osteomyelitis patients according to gender, hemoglobin profile (HbS, HbF), and other hematological indices (Table 1). The local research and ethics committees approved the study protocol, which was in agreement with the Helsinki declaration of 2000. All participants (or guardians, in the pediatric cases) gave written informed consent after the purpose of the study was explained to them.

**Results**

The demographic and clinical characteristics of the study participants are shown in Table 1. The osteomyelitis group consisted of 52 SCD patients with confirmed diagnosis of osteomyelitis, while the control group consisted of 165 age-matched (P = 0.118) and gender-matched (P = 0.268) SCD subjects with negative histories of osteomyelitis. With the exception of platelet count (P = 0.039), all biochemical and hematological indices were comparable between the two SCD patient groups. The mean IL-10 serum value in osteomyelitis SCD patients was 15.9 ± 6.4 pg/ml, which was significantly lower than that of control SCD individuals (27.2 ± 17.8 pg/ml) (P < 0.001). In addition, there was no association between the nature of the bacterial infection (Salmonella versus Staphylococcus) and serum IL-10 levels (P = 0.254).

Receiver operating characteristic (ROC) analysis was performed to determine the predictive value of IL-10 levels in the prediction of osteomyelitis (Fig. 1). Area under ROC curves provided good discriminatory power for IL-10 and osteomyelitis and demonstrated that altered IL-10 serum levels displayed comparable sensitivities and specificities for predicting the development of osteomyelitis. Spearman’s correlation coefficient between IL-10 and osteomyelitis was −0.308 (P = 3.2 × 10^{-6}) among unselected patients, −0.490 (P = 3.0 × 10^{-8}) in males, and −0.414 (P = 8.1 × 10^{-7}) in females. The area under ROC curves of IL-10 was 0.810 ± 0.034 (95% CI, 0.742 to 0.877) among unselected patients, 0.829 ± 0.043 (95% CI, 0.744 to 0.914) for males, and 0.787 ± 0.059 (95% CI, 0.671 to 0.903) for females.

A systematic shift in IL-10 serum level distributions toward lower values was noted in osteomyelitis cases. IL-10 levels were then categorized into 5 strata: percentiles 1 to 25 (P25), 26 to 50 (P50), 51 to 75 (P75), 76 to 90 (P90), and >90 (P95), according to

---

**Table 1. Characteristics of study participants**

| Characteristic | Osteomyelitis | Control SCD | P |
|---------------|--------------|-------------|---|
| Age (yr)      | 10.1 ± 5.7   | 12.5 ± 12.8 | 0.118 |
| No. of male/female participants | 32/19 | 99/66 | 0.268 |
| Hb profile (%) |              |             |    |
| HbS           | 71.7 ± 10.9  | 71.2 ± 8.3  | 0.800 |
| HbF           | 18.1 ± 6.6   | 19.6 ± 7.6  | 0.232 |
| Total hemoglobin (g/dl) | 9.4 ± 1.2 | 9.5 ± 1.3 | 0.252 |

Hematological indices:

| Characteristic | Osteomyelitis | Control SCD | P |
|---------------|--------------|-------------|---|
| WBC (×10^9/liter) | 9.7 ± 5.0 | 10.2 ± 4.0  | 0.417 |
| Platelets (×10^9/liter) | 388.1 ± 239.8 | 315.6 ± 172.6 | 0.039 |
| Hematocrit (%) | 29.6 ± 4.6 | 27.7 ± 6.4  | 0.268 |
| Mean corpuscular vol (fl) | 75.6 ± 9.8 | 76.6 ± 10.4 | 0.605 |
| Mean corpuscular Hb (pg) | 25.7 ± 4.0 | 26.2 ± 5.6  | 0.573 |
| Mean corpuscular Hb concn (g/dl) | 33.6 ± 2.7 | 34.0 ± 1.6  | 0.451 |
| Reticulocytes (%) | 5.3 ± 3.8 | 4.9 ± 3.3   | 0.540 |
| IL-10 (pg/ml) | 15.9 ± 6.4  | 27.2 ± 17.8 | 1.3 × 10^{-5} |

---

*Osteomyelitis group: n = 52 patients with SCD and osteomyelitis. Except for the number of male/female participants, data indicate mean values ± SD.
*Control group: n = 165 patients with SCD without history of osteomyelitis. Except for the number of male/female participants, data indicate mean values ± SD.
*Pearson’s χ² test for categorical variables and two-tailed t test for continuous variables.
IL-10 serum levels present in the control group. Categorized IL-10 levels were analyzed in regression models, first at the univariate level and later at the multivariate level. Univariate regression analysis demonstrated a positive dose-effect relationship for IL-10 with osteomyelitis, with increased osteomyelitis risk associated with decreased IL-10 levels (Table 2). The strongest OR was for the IL-10 P90 percentile, which was associated with a 21.74-fold higher risk than the P25 percentile (Table 2).

Given the shift in the IL-10 cutoff values between osteomyelitis and control SCD patients, for which significant differences were seen at the P25 and >P75 percentiles, IL-10 levels in SCD patients were then categorized by threshold limits corresponding to low (<18.24 pg/ml), intermediate (18.25 to 30.00 pg/ml), and high (>30.00 pg/ml) percentiles according to concentrations present in the SCD control group. Setting the lower values as a reference (OR, 1.00), monovariate logistic regression analyses demonstrated a dose-effect relationship for IL-10 with osteomyelitis, with an increased osteomyelitis risk associated with decreased IL-10 serum levels (Table 2). Multivariate logistic regression analyses confirmed the independent association of reduced IL-10 with osteomyelitis (Table 3). None of the variables entered in the model (HbS, HbF, platelet count, and WBC count) was found to be associated with osteomyelitis (Table 3).

**DISCUSSION**

We recently demonstrated a strong association of reduced IL-10 levels with VOC and modulation of VOC-related parameters, namely type, frequency, severity, and duration of VOC, in pediatric SCD patients (25). VOC, and associated osteomyelitis, is a common acute clinical manifestation in children with SCD. Osteomyelitis presents as an acute or chronic infection of the bone and is characterized by supplicative inflammation and abnormal bone remodeling, together with uncontrolled bone resorption (26). IL-10 acts largely as an anti-inflammatory cytokine; given that inflammatory changes accompany osteomyelitis in SCD, this suggests a role for IL-10 in the pathogenesis of osteomyelitis. To the best of our knowledge, no previous study has examined changes in serum IL-10 levels in SCD osteomyelitis.

VOC and osteomyelitis have unique clinical presentations in the acute stages of SCD, thereby necessitating careful diagnosis. To investigate the relationship between low IL-10 levels and osteomyelitis in pediatric SCD patients, samples were collected based on clinical assessment and positive radiological findings, along with hematological analysis (erythrocyte sedimentation rate and C-reactive protein), blood culture, and bone or joint pus aspiration. Age-matched and ethnically matched pain-free non-osteomyelitis SCD controls were included during active osteomyelitis episodes, so as to minimize the variability inherent in soluble cytokine determination. The obtained results demonstrated a significant association between reduced IL-10 levels and osteomyelitis development, which was confirmed by the enrichment of low IL-10 producers in osteomyelitis cases compared with control SCD patients.

The high level of proinflammatory cytokines during osteomyelitis can be attributed to frank upregulation of proinflammatory cytokine expression and/or the absence or low level of anti-inflammatory factors, including IL-10. IL-10 is a Th2 cytokine with immunomodulatory functions and broad-spectrum anti-inflammatory activity in various models of infections, inflammation, and some cancers. The immune-inhibitory capacity of IL-10 is largely due to its effect on antigen-presenting cells and on the production of the Th1 cytokines, notably IL-2 and gamma interferon (IFN-γ), and also to the induction of the Th2 cytokines IL-4 and IL-5 (27–30). The major inflammatory cytokines, IL-1, IL-6, IL-12, and TNF, are dramatically repressed following exposure to IL-10. IL-10 can further inhibit inflammation by increasing the release of an IL-1 receptor antagonist by macrophages.

Altered cytokine expression in infected bones, associated bone resorption, and higher osteoclast activities are hallmark features of osteomyelitis in pediatric SCD patients (25). VOC, and associated osteomyelitis, is a common acute clinical manifestation in children with SCD. Osteomyelitis presents as an acute or chronic infection of the bone and is characterized by supplicative inflammation and abnormal bone remodeling, together with uncontrolled bone resorption.
osteomyelitis (17, 31–34). However, the exact mechanisms by which cytokines mediate the changes associated with osteomyelitis are not completely known. Our data support the notion that reduced IL-10 secretion augments inflammatory changes seen in osteomyelitis. While no previous study examined the association of IL-10 with SCD osteomyelitis, recent studies suggest temporal changes in cytokine secretion during the course of osteomyelitis. Early osteomyelitis episodes were accompanied by an increased frequency of high TNF-α and IL-4 producers, whereas late osteomyelitis events are characterized by increased frequencies of high IL-4, IL-10, IL-6, and IL-2 producers (11). Reduced IL-10 expression was associated with experimental chronic Staphylococcus aureus–induced osteomyelitis in a rodent model, and improvement in the course of the osteomyelitis resulting from antibiotic treatment was linked with increased IL-10 expression (35).

Mechanistically, IL-10 suppresses infection-stimulated bone resorption in vivo by inhibiting IL-1α expression, with reduced/absent IL-10 expression associated with a compensatory mechanism involving increased IL-6 secretion being operational in reducing inflammation (36). IL-10 production is racially restricted and varies markedly among individuals, thus prompting the speculation that low IL-10 producers are at a higher risk of developing inflammatory diseases. However, in chronic progressive inflammatory conditions, including osteomyelitis, a secondary reactive loss of IL-10 appears rather likely and, thus, cannot be overlooked.

While our study clearly demonstrated an association between reduced IL-10 secretion and risk of osteomyelitis in SCD patients, some limitations to these findings are noteworthy. IL-10 levels were measured following osteomyelitis, which raises the possibility that they were the consequence, but not the cause, of osteomyelitis. Another shortcoming was our selection of VOC patients, since only pediatric cases were included, which necessitates performing parallel determinations on adult SCD patients with osteomyelitis. Despite these shortcomings, our results support the notion of a covert inflammatory response in osteomyelitis which will be instrumental in the future management strategies of osteomyelitis episodes.

ACKNOWLEDGMENT
We declare no competing financial interests.

REFERENCES
1. Sheth S, Licursi M, Bhatia M. 2013. Sickle cell disease: time for a closer look at treatment options? Br J Haematol 162:455–464. http://dx.doi.org/10.1111/bjh.12413.
2. Alsultan A, Aleem A, Ghabbour H, AlGahtani FH, Al-Shehri A, Osman ME, Kurban K, AlSulata MS, Bahakim H, Al-Momen AM. 2012. Sickle cell disease subphenotypes in patients from southwestern province of Saudi Arabia. J Pediatr Hematol Oncol 34:79–84. http://dx.doi.org/10.1097/MPH.0b013e3182242284.
3. Almeida A, Roberts I. 2005. Bone involvement in sickle cell disease. Br J Haematol 129:482–490. http://dx.doi.org/10.1111/j.1365-2141.2005.05476.x.
4. Ganguly A, Boswell W, Aniq H. 2011. Musculoskeletal manifestations of sickle cell anemia: a pictorial review. Anemia 2011:794283.
5. Inusa BP, Oyewo A, Brokke F, Santhikumaran G, Jogeesevan KH. 2013. Dilemma in differentiating between acute osteomyelitis and bone infarction in children with sickle cell disease: the role of ultrasound. PTOS One 8:e65001. http://dx.doi.org/10.1371/journal.pone.0065001.
6. Ejindu VC, Hine AI, Mashayekhi M, Shorvon PJ, Misra RR. 2007. Musculoskeletal manifestations of sickle cell disease. Radiographics 27:1005–1021. http://dx.doi.org/10.1148/rg.2740651412.
7. Akapko-Numado GK, Gnassingsbe K, Boume MA, Songne B, Tekou H. 2008. Current bacterial causes of osteomyelitis in children with sickle cell disease. Sante 18:67–70. http://dx.doi.org/10.1684/san.2008.0106.
8. Al-Ola K, Mahdi N, Al-Subaie ME, Ali ME, Al-Abri IK, Almawi WY. 2008. Evidence for HLA class II susceptible and protective haplotypes for osteomyelitis in pediatric patients with sickle cell anemia. Tissue Antigens 71:453–457. http://dx.doi.org/10.1111/j.1399-0039.2008.01012.x.
9. Tseou A, Poultside L, Kostopoulos F, Zintzaras E, Satra M, Kitisou-Tzeli S, Malizos KN. 2008. Influence of interleukin 1 (IL-1), IL-4, and IL-6 polymorphisms on genetic susceptibility to chronic osteomyelitis. Clin Vaccine Immunol 15:1888–1890. http://dx.doi.org/10.1128/CVI.00209-08.
10. Berger E, Saunders N, Wang I, Friedman JN. 2009. Sickle cell disease in children: differentiating osteomyelitis from vasoocclusive crisis. Arch Pediatr Adolesc Med 163:251–255. http://dx.doi.org/10.1001/archpediatrics.2008.545.
11. Ferreira GF, Moraes C, Silveira AM, Friedman JN. 2012. Distinct cytokine profiles of circulating mononuclear cells stimulated with Staphylococcus aureus enterotoxin A in vitro during early and late episodes of chronic osteomyelitis. Mem Inst Oswaldo Cruz 107:348–355. http://dx.doi.org/10.1590/S0074-02762012000300009.
12. Klosterhalfen B, Peters KM, Tons C, Hauptmann S, Klein CL, Kirkpatrick CJ. 1996. Local and systemic inflammatory mediator release in patients with acute and chronic posttraumatic osteomyelitis. J Trauma 40:372–378. http://dx.doi.org/10.1097/00005373-199603000-00008.
13. Evans CA, Jelhis J, Hughes SP, Remick DG, Friedland JS. 1998. Tumor necrosis factor-alpha, interleukin-6, and interleukin-8 secretion and the acute-phase response in patients with bacterial and tuberculous osteomyelitis. J Infect Dis 177:1582–1587. http://dx.doi.org/10.1086/515313.
14. Balto K, Sasaki H, Stashenko. 2001. Interleukin-6 deficiency increases inflammatory bone destruction. Infect Immun 69:744–750. http://dx.doi.org/10.1128/IAI.69.2.744-750.2001.
15. Fullilove S, Jelhis J, Hughes S, Remick DG, Friedland JS. 2000. Local and systemic concentrations of tumor necrosis factor-α, interleukin-6, and interleukin-8 in bacterial osteomyelitis. Trans R Soc Trop Med Hyg 94:221–224. http://dx.doi.org/10.1016/S0033-9203(00)90284-0.
16. Marriott I, Gray DL, Tranugch SL, Fowler VG, Jr, Stryjewski M, Scott Levin L, Hudson MC, Bost KL. 2004. Osteoblasts express the inflammatory cytokine interleukin-6 in a murine model of Staphylococcus aureus osteomyelitis and infected human bone tissue. Am J Path 164:1399–1406. http://dx.doi.org/10.1016/S0002-9440(10)6226-9.
17. Pesanti EL, Lorenzo JA. 1998. Osteoclasts and effects of interleukin in development of chronic osteomyelitis. Clin Orthop Relat Res 355:290–299. http://dx.doi.org/10.1097/00003086-199810000-00031.
18. Cronstein BN. 2007. Interleukin-6: a key mediator of systemic and local symptoms in rheumatoid arthritis. Bull NYU Hosp Jt Dis 65(Suppl 1):S11–S15.
19. Hart PH, Jones CA, Finlay-Jones J. 1995. Monocytes cultured in cyto-

### TABLE 3 Regression analysis of IL-10 in SCD osteomyelitis patients

| IL-10 serum level (pg/ml) | Osteomyelitis | SCD controls | P       | aOR* | 95% CI       |
|---------------------------|---------------|--------------|---------|------|-------------|
| 0 to <18.2                | 36 (69.2)     | 43 (25.6)    | 4.8 × 10^-5 | 1.00 |             |
| ≥18.2 to <30.0            | 14 (26.9)     | 69 (41.1)    | 0.021   | 7.47 | 1.55 to 35.91 |
| ≥30.0                     | 2 (3.8)       | 56 (33.3)    | 2.7 × 10^-4 | 17.25 | 3.72 to 79.99 |

* aOR, adjusted odds ratio. Covariates controlled for were platelet and WBC counts and HbS and HbF levels.
kine-defined environments differ from freshly isolated monocytes in their responses to IL-4 and IL-10. J Leukoc Biol 57:909–918.

20. Wang P, Wu P, Siegel MI, Egan RW, Billah MM. 1995. Interleukin (IL)-10 inhibits nuclear factor κB (NF-κB) activation in human monocytes: IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. J Biol Chem 270:9558–9563. http://dx.doi.org/10.1074/jbc.270.16.9558.

21. Yoshii T, Magara S, Miyai D, Kuroki E, Furudoi S, Komori T, Ohbayashi C. 2002. Local levels of interleukin-1β,-4,-6 and tumor necrosis factor α in an experimental model of murine osteomyelitis due to staphylococcus aureus. Cytokine 19:95–65. http://dx.doi.org/10.1006/cyto.2002.1039.

22. Zhang X, Teng YT. 2006. Interleukin-10 inhibits gram-negative-microbe-specific human receptor activator of NF-κB ligand-positive CD4⁺-Th1-cell-associated alveolar bone loss in vivo. Infect Immun 74:4927–4931. http://dx.doi.org/10.1128/IAI.00491-06.

23. Sabat R, Grutz G, Warszawska K, Kirsch S, Witte E, Wolk K, Geginat J. 2010. Biology of interleukin-10. Cytokine Growth Factor Rev 21:331–344. http://dx.doi.org/10.1016/j.cytogfr.2010.09.002.

24. Le Saux N, Howard A, Barrowman NJ, Gaboury I, Sampson M, Moher D. 2002. Shorter courses of parenteral antibiotic therapy do not appear to influence response rates for children with acute hematogenous osteomyelitis: a systematic review. BMC Infect Dis 2:16. http://dx.doi.org/10.1186/1471-2334-2-16.

25. Sarray N, Howard A, Barrowman NJ, Gaboury I, Sampson M, Moher D. 2002. Shorter courses of parenteral antibiotic therapy do not appear to influence response rates for children with acute hematogenous osteomyelitis: a systematic review. BMC Infect Dis 2:16. http://dx.doi.org/10.1186/1471-2334-2-16.

26. Sarray N, Mahdi N, Saleh LR, Almaoui WY. 2014. Reduction in serum IL-10 levels is a surrogate marker for predicting vasocclusive crisis in sickle cell disease. Am J Hematol 89:789–790. http://dx.doi.org/10.1002/ajh.23736, 10.1002/ajh.23748.

27. Montanaro L, Testoni F, Poggia F, Visai L, Speziale P, Arciola CR. 2011. Emerging pathogenetic mechanisms of the implant-related osteomyelitis by Staphylococcus aureus. Int J Artif Organs 34:781–788. http://dx.doi.org/10.5301/ijao.5000052.

28. Santin AD, Hermonat PL, Ravaghi A, Bellone S, Pecorelli S, Roman J, Parham GP, Cannon MJ. 2000. Interleukin-10 increases Th1 cytokine production and cytotoxic potential in human papillomavirus-specific CD8⁺ T lymphocytes. J Virol 74:4729–4937. http://dx.doi.org/10.1128/JVI.74.10.4729-4737.2000.

29. Rowbottom AW, Lepper MA, Garland RJ, Cox CV, Corley EG. 1999. Interleukin-10-induced CD8 cell proliferation. Immunology 98:80–89. http://dx.doi.org/10.1046/j.1365-2567.1999.00828.x.

30. Groux H, Bigler M, de Vries JE, Roncarolo MG. 1998. Inhibitory and stimulatory effects of IL-10 on human CD8⁺ T cells. J Immunol 160:3188–3193.

31. Lauw FN, Pajkrt D, Hack CE, Kurimoto M, van Deventer SJ, van der Poll T. 2000. Proinflammatory effects of IL-10 during human endotoxemia. J Immunol 165:2783–2789. http://dx.doi.org/10.4049/jimmunol.165.5.2783.

32. Asensi V, Alvarez V, Valle E, Meana A, Fierer J, Coto E, Carton JA, Maradona JA, Paz J, Dieguez MA, de la Fuente B, Moreno A, Rubio S, Tuya MJ, Sarasúa J, Llanes S, Arribas JM. 2003. IL-10 (-889) promoter polymorphism is a risk factor for osteomyelitis. Am J Med Genet A 119A:132–136. http://dx.doi.org/10.1002/ajmg.a.20137.

33. Asensi V, Valle E, Meana A, Fierer Fierer J, Celada A, Alvarez V, Paz J, Coto E, Carton JA, Maradona JA, Dieguez A, Sarasúa J. Ocaña MG, Arribas JM. 2004. In vivo interleukin-6 protects neutrophils from apoptosis in osteomyelitis. Infect Immun 72:3823–3828. http://dx.doi.org/10.1128/IAI.72.7.3823-3828.2004.

34. Kwan Tat S, Padrines M, Theoleyre S, Heymann D, Fortun Y. 2004. IL-6, RANKL, TNF α/IL-1: interrelations in bone resorption pathophysiology. Cytokine Growth Factor Rev 15:49–60. http://dx.doi.org/10.1016/j.cytogfr.2003.10.005.

35. Miossec P, Chomarat P, Dechanet J, Moreau JF, Roux JP, Delmas P, Banchereau J. 1994. Interleukin-4 inhibits bone resorption through an effect on osteoclasts and proinflammatory cytokines in an ex vivo model of bone resorption in rheumatoid arthritis. Arthritis Rheum 37:1715–1722. http://dx.doi.org/10.1002/art.1780371202.

36. García-Alvarez I, Navarro-Zorraquino M, Alvarez FG. 2000. IL-10, but not IL-4, suppresses infection-stimulated bone resorption in experimental chronic Staphylococcus aureus osteomyelitis. J Orthop Sci 11:370–374. http://dx.doi.org/10.1007/s00776-001626-9.

37. Sasaki H, Hou L, Belani A, Wang CY, Uchiyama T, Müller R, Stashenko P. 2000. IL-10, but not IL-4, suppresses infection-stimulated bone resorption in vivo. J Immunol 165:3626–3630. http://dx.doi.org/10.4049/jimmunol.165.7.3626.

38. Woolf SH. 2000. Taking critical appraisal to extremes. The need for balance in the evaluation of evidence. J Fam Pract 49:1081–1085.