

T-CELL CO-STIMULATORS IN OSTEOLYSIS FOLLOWING TOTAL HIP REPLACEMENT

MAHITO KAWASHIMA, PHD, K HAYATA, PHD, HARRY E. RUBASH, MD, AND ARUN S. SHANBHAG, PHD

BIOMATERIALS LAB, MASSACHUSETTS GENERAL HOSPITAL

INTRODUCTION

Interfacial tissues surrounding failed joint replacements have provided crucial insight into the mechanisms of periprosthetic bone loss and implant loosening. Using high throughput protein chips, we recently reported high levels of T-cell chemokines: interferon- γ -inducible protein of 10KDa (IP-10) and monokine induced by interferon- γ (MIG) in osteolytic tissues (1). The question posed is: if activated T-cells are recruited to the inflammatory site, are other co-stimulators and modulators of antigen presentation and immunological synapse formation (B7-1, B7-2 and CD-28) also upregulated? We tested for the gene expression of these modulators, as well as IP-10, MIG and cytotoxic T-lymphocyte antigen 4 (CTLA-4) using RT-PCR.

MATERIALS AND METHODS

CLINICAL

Interfacial tissues were harvested from patients (n=14; mean age = 66y) undergoing revision surgery for aseptic loosening of their femoral total hip replacement (THR). Capsular tissues from patients with end-stage osteoarthritis (OA) undergoing primary THR (n=14, mean age = 67y) provided control comparisons. In the operating room, tissues were flash frozen in liquid nitrogen and subsequently homogenized and had their mRNA extracted.

RNA EXTRACTION AND RT-PCR

Total RNA was extracted from 1cm³ of periprosthetic tissue by homogenization in 1.6mL TRIzol reagent (Invitrogen, Paisley, UK). RNA was extracted using established protocol and cleaned using RNeasy[®] Mini kit (Qiagen, Valencia, CA). RNA samples were stored at (-)76 °C.

Mahito Kawashima Ph.D. is a member of the Biomaterials Lab at Massachusetts General Hospital.

K Hayata Ph.D. is a member of the Biomaterials Lab at Massachusetts General Hospital.

Harry E. Rubash M.D. is Chief of the Orthopedic Department at Massachusetts General Hospital.

Arun S. Shanbhag Ph.D., MBA is Director of the Biomaterials Lab at Massachusetts General Hospital and Assistant Professor of Orthopedic Surgery at Harvard Medical School.

Address correspondence to:

Arun Shanbhag, Ph.D, MBA
GRJ 1115, 55 Fruit St.
Boston, MA 02114
shanbhag@helix.mgh.harvard.edu

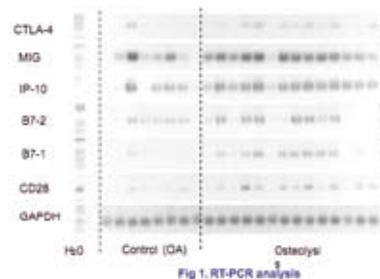


Figure 1: Gene expression of CTLA-4, MIG, IP-10, B7-1, B7-2 and CD28 using RT-PCR. GAPDH was used as a housekeeping gene.

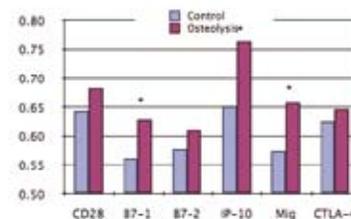


Figure 2: Intensity value analysis of CTLA-4, MIG, IP-10, B7-1, B7-2 and CD28 using RT-PCR (mean +/- SE, *p < 0.05 over control).

PCR amplification was performed in 50 μ l reactions containing 20 μ l Eppendorf[®] Master Mix (2.5x) (Eppendorf AG, Hamburg, Germany), 2 μ l cDNA and each oligonucleotide primer at 0.5 μ M. Oligonucleotide primers were designed according to previously published sequences for IP-10 (2), MIG (3), B7-1/CD80 and B7-2/CD86 (4), CD28 (5), and CTLA-4/CD152 (6). GAPDH was used as a housekeeping gene to test integrity of sample cDNA. Data was analyzed using both the F statistic and Student's t-test. A p-value of < 0.05 was considered significant.

RESULTS

Compiled PCR results are shown in Figure 1 and the intensity values are plotted in Figure 2. In most cases of implant loosening significant levels of MIG and IP-10 were detected. This finding concurs with our protein studies indicating significant release of these two important T-cell chemokines (1). We also detected strong expression of T-cell modulators: B7-1, B7-2, CD28 in osteolysis samples and to a lesser extent in OA cases. Interestingly, CTLA-4, a repressor of the T-cell activation, was also upregulated in a few osteolysis cases (not significant).

DISCUSSION

IP-10 and MIG are produced by IFN- γ stimulated monocytes, macrophages and antigen experienced T cells and are a chemoattractant for activated Th1 cells and natural killer (NK) cells. They also share common receptor. In this work we have demonstrated that these higher protein levels are associated with a concomitant upregulation of gene expression. These

findings suggest that antigen is being presented and activated T-cells are indeed recruited to the inflammatory site in osteolysis. Additional co-stimulators such as B7-1, B7-2 and CD28 are also upregulated – these are required for the development of the immunological synapse between the antigen-presenting cell (APC) and the T-cell. B7-1 and B7-2 have largely overlapping functions. They provide important co-stimulatory signals to augment and sustain a T cell response via an interaction with CD28. B7-2 is expressed constitutively at low levels on APCs and is rapidly upregulated in immune responses, whereas B7-1 is inducibly expressed later after activation. In the present study, we demonstrated a significantly higher level of B7-1 gene expression in osteolytic tissues, compared with B7-2 expression. These results suggest that osteolysis may represent a late stage in the immune response.

Because important T-cell mediators such as IL-2, INF- γ , IL-1, and TNF- α are not detected in protein arrays, the immune response appears to have been aborted (1). Notably, mRNA expression of CTLA-4 is upregulated in some cases of osteolysis. CTLA-4 is an integrin expressed on activated T-cells and

inhibits IL-2 secretion from T-cells (7). This is additionally supported by high protein levels of soluble intercellular adhesion molecule-1 (sICAM-1) which serves as a soluble receptor for the leukocyte integrin, lymphocyte function-associated antigen (LFA-1)/CD18. While LFA-1 facilitates leukocyte adhesion and migration across the endothelium, sICAM-1 binding to LFA-1 disrupts the immune synapse and prevents propagation of a robust T-cell mediated immune response (8).

SUMMARY

T-cell co-stimulators, CD28, B7-1 and B7-2 are crucial for facilitating and stabilizing the immunological synapse, and are expressed in osteolytic tissues. While the underlying mechanisms are still being clarified, these results indicate that the participation of an immunological reaction, alongside the well described macrophage-mediated response to foreign body, is important in leading to osteolysis and aseptic loosening of total joint replacements.

Acknowledgements: MGH Orthopaedics for financial support

References

1. **Shanbhag AS, Kaufman AM, Agarwal S, et al.** Critical Insights into osteolysis using protein microarrays: The importance of IL-6 and T-cell Activation. *Trans Orthop Res Soc* 2005; 30:148.
2. **Gasper NA, Petty CC, Schrum LW, et al.** Bacterium-induced CXCL10 secretion by osteoblasts can be mediated in part through toll-like receptor 4. *Infect Immun.* 2002 Aug;70(8):4075-82.
3. **Raju,R, Malloy,A, Shah,T, et al.** Alloimmune induction of endothelial cell-derived interferon-gamma-inducible chemokines: *Transplantation.* 75:1072-4 (2003)
4. **Kiefer R, Dangond F, Mueller M, et al.** Enhanced B7 costimulatory molecule expression in inflammatory human sural nerve biopsies: *J Neurol Neurosurg Psychiatry.* 69: 362-368 (2000)
5. **Xu KL, Zhang Y, Pan XY, Lu QX.** Inhibiting the expression of CD28 costimulatory molecule on human lymphocytes by special siRNA: *Clin Med J.* 118: 480-486 (2005)
6. **Murata K, Dalakas MC.** Expression of the costimulatory molecule BB-1 the ligands CTLA-4 and CD28 and their mRNA in inflammatory myopathies: *Am J Pathol.* 155:453-60 (1999)
7. **Walunas TL, Lenschow DJ, Bakker CY, et al.** CTLA-4 can function as a negative regulator of T cell activation: *Immunity.* 1: 405-413 (1994).
8. **Rieckmann P, Michel U, Albrecht M, et al.** Soluble forms of intercellular adhesion molecule-1 (ICAM-1) block lymphocyte attachment to cerebral endothelial cells: *Neuroimmunol.* 60: 9-15 (1995).