Analysis of Peripheral Blood Lymphocyte Phenotypes in Patients with Failed Metal-on-Metal Hip Implants

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Introduction:

A major cause for concern with metal-on-metal (MM) hip implants has emerged because of increasing reports of early adverse tissue reactions, including soft tissue masses, also referred to as pseudotumors [1]. Pseudotumor histology includes features consistent with non-specific inflammatory reactions to metal wear such as macrophages with particles, but also features consistent with a specific metal hypersensitivity immune reaction such as lymphocyte aggregates [2]. Pseudotumors have been associated with high wear and low hypersensitivity, and vice-versa [2]. Hence, their exact causes and mechanisms remain unknown, but the presence of lymphocyte aggregates points towards a role for the adaptive immune response and a hypersensitivity reaction. Implant-related hypersensitivity reactions are thought to be T-lymphocyte cell-mediated, type-IV hypersensitivity reactions [3]. Hence, if a type-IV hypersensitivity reaction is prevalent in patients with failed MM implants associated with a pseudotumor, a local increase in memory T-lymphocyte percentages would be expected. Furthermore, since type 1 T-helper (Th) cells (expressing interferon-gamma, IFN-γ) are involved in type-IV hypersensitivity reactions, local increased proportions of IFN-γ-expressing Th cells would also be expected. Therefore, the objective of this study was to compare the proportions of lymphocyte subtypes in peripheral blood from two groups of patients: those with failed MM hip implants associated with a pseudotumor and those with MM hip implants that failed because of other reasons. This comparison will reveal potential differences in the systemic immune response which would reflect differences in the local periprosthetic tissues.

Materials and Methods:

This study has been approved by the Ottawa Hospital Research Ethics Boards. Heparinized peripheral blood samples were obtained from consenting patients prior to revision surgery of failed MM implants associated with a pseudotumor (2 males and 4 females, 60.8 ± 9.6 years old, mean time to failure of 3.6 ± 0.8 years) and revision surgery of MM implants that failed because of other reasons (15 males and 6 females, 52.7 ± 10.0 years old, mean time to failure of 2.9 ± 1.5 years).

Peripheral blood mononuclear cells were isolated by Ficoll density gradient, and stained for surface markers of T-cells (CD3, CD4 (T-helper (Th)) and CD8 (T-cytotoxic (Tc))), B-cells (CD19) and natural killer (NK) cells (CD56), as well as for surface markers of memory T- and B-cells (CD45RO and CD27, respectively). Isolated cells were also cultured in 24-well plates for 5.5 hours in the presence of phorbol-12-myristate-13-acetate (PMA), ionomycin and brefeldin A. Following incubation, cells were stained for surface markers (CD3 and CD4), fixed, permeabilized, and stained for IFN-γ and interleukin-4 (IL-4) to measure the percentages of Th cells and $CD3^+CD4^-$ cells (considered to be primarily $CD8^+$ T-cells, i.e., Tc cells), expressing IFN-γ and IL-4. The stained cells were then analyzed by flow cytometry to determine the percentages of each lymphocyte subtype. The Shapiro-Wilk test and F-test were used to analyze normality in data distributions and differences in group variances, respectively. When the distributions were not normal, statistical analysis was performed using the Mann Whitney U test. Otherwise, a Student's t-test was used (no Welch correction needed to be applied since variances were equal in all cases). A p-value <0.05 was considered significant.

Results:

No significant difference was observed between the two experimental groups for T-, B- and NK-cells ($CD3^+$, $CD19^+$, CD56+, respectively), nor for the ratios of $CD4^+/CD8^+$ T-cell percentages (data not shown). However, the mean percentages of total memory T-cells ($CD45RO^+$) and specifically memory $CD4^+$ and memory $CD8^+$ cells were significantly lower in the MM group with pseudotumors (p=0.0006, p=0.0003 and 0.0025, respectively) (Fig. 1A and B). On the other hand, there was no significant difference in the mean percentages of total memory B-cells.

The analysis of intracellular cytokine expression revealed a significant difference between the two experimental groups for total T-cells expressing IFN-γ (p=0.0022) but not for total T-cells expressing IL-4. Specifically, results showed a significant difference for both $CD3^+CD4^+$ cells (Th-cells) and $CD3^+CD4^-$ cells (considered to be primarily Tc cells) expressing IFN-γ (p<0.0001 and 0.0283, respectively). Overall, the MM group with pseudotumors exhibited lower percentages (Fig. 1C and 1D). Finally, the percentages of $CD3^+CD4^+$ and $CD3^+CD4^-$ T-cells expressing IL-4 remained low, and the two groups were not significantly different (data not shown).

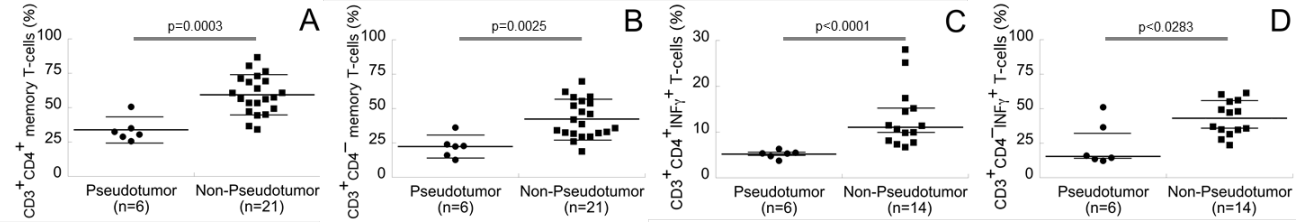

Figure 1: Subtypes of peripheral blood T lymphocytes. Vertical scatter plots show means ± SD (A and B) and median with interquartile ranges (C and D). Data are presented as percentages of T cells that are: (A) $CD3^+CD4^+$; (B) $CD3^+CD4^-$; (C) $CD3^+CD4^+IFN\gamma^+$; and (D) $CD3^+CD4^-IFN\gamma^+$.

Discussion:

Overall, this study shows significant differences in the proportions of lymphocyte subtypes, particularly in memory T-cells as well as type 1 Th and Tc cells (cells expressing IFN-γ), in peripheral blood of patients with failed MM implants associated with a pseudotumor compared to other MM patients. Specifically, results showed lower proportions of memory T-cells in patients with a pseudotumor. This suggests a lower number of memory T-cells circulating systemically, which could reflect a sequestration of these cells in periprosthetic tissues (i.e., at the local site of the adverse reaction). However, this would need to be confirmed with absolute cell counts. A local increase in the number of memory T-cells in the tissues would be consistent with a type-IV hypersensitivity reaction. Results also showed lower proportions of type 1 Th and Tc cells in the MM group with pseudotumors. Similarly to the memory T-cells results, this may reflect a sequestration of type 1 T-cells in periprosthetic tissues, which would also be consistent with a type-IV hypersensitivity reaction.

Overall, results show specific phenotypic differences in MM patients with a pseudotumor compared to other MM patients. These differences could potentially become diagnostic markers for the detection of this type of adverse tissue reaction. Nevertheless, group sizes need to be increased to confirm the observed phenotypic differences and results should be correlated to histological analysis of periprosthetic tissues.

References: 1. Pandit et al. (2008) *J Bone Joint Surg* (Br) 90(7): 847-51; 2. Campbell et al. (2010) *Clin Orthop Rel Res* 468(9), 2321-27; 3. Goodman S.B. (2007) *Biomaterials* 28(34): 5044-48.

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