Introduction: Early adverse tissue reactions, including soft tissue masses, also referred to as pseudotumors [1], have become a major concern with metal-on-metal (MM) hip implants. 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. Metal implant-related hypersensitivity reactions were reported 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 hip implants associated with a pseudotumor, a local increase in memory T-lymphocytes would be expected. Furthermore, since type 1 T-helper (Th1) cells (Th cells expressing interferon-gamma, IFN-γ) are involved in type-IV hypersensitivity reactions, a local increase in IFN-γ-expressing Th cells would also be expected. Therefore, the objective of this study was to compare the proportions of lymphocyte subpopulations in peripheral blood from three groups of patients with MM hip implants: patients with failed implants with a pseudotumor; patients with failed implants without a pseudotumor; and patients with well-functioning implants. This comparison will reveal potential differences in the systemic immune response that are expected to reflect local differences in the periprosthetic tissues.

Methods: This study has been approved by the Ottawa Hospital Research Ethics Boards. Heparinized peripheral blood samples were obtained from consenting patients with failed MM hip implants with a pseudotumor (2 males and 4 females, 58.4 ± 13.6 years old, mean time to failure of 3.3 ± 0.8 years), patients with failed MM hip implants without a pseudotumor (12 males and 6 females, 54.4 ± 10.3 years old, mean time to failure of 3.4 ± 1.7 years), and patients with well-functioning MM hip implants at >5 years (18 males and 6 females; 58.2 ± 11.6 years old; mean time of implantation of 6.1 ± 1.0 years). Peripheral blood mononuclear cells were isolated by 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 CD3+CD4+ (Th cells) and CD3+CD8- 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 subpopulation. The Shapiro-Wilk and Bartlett tests were used to analyze normality in data distributions and differences in group variances, respectively. When the distributions were normal with no significant differences in group variances, statistical analysis was performed using ANOVA and Tukey-Kramer tests. When the distributions were not normal, statistical analysis was performed using the Kruskal Wallis and two-sided Mann-Whitney U tests. A p-value <0.05 was considered significant.

Results: No significant differences were observed between the three 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 and, specifically, memory CD4+ (Th) cells and memory CD8+ (Tc) cells were significantly lower in the group with failed MM hip implants with a pseudotumor than in the group with failed implants without a pseudotumor and the group with well-functioning implants (p<0.03 in all cases) (Fig. 1A and B). For memory B-cells, a significant difference was observed between the group with failed MM hip implants without a pseudotumor and the other two groups (p<0.04 in both cases), with overall higher percentages in the group with failed implants without a pseudotumor (data not shown). The analysis of intracellular cytokine expression revealed a significant difference between the three experimental groups for total T-cells expressing IFN-γ (p=0.030), but not for total T-cells expressing IL-4. For CD3+CD4+ cells expressing IFN-γ (type 1 Th cells), a significant difference was observed between the three experimental groups (p=0.018) and, specifically, between the group with failed MM hip implants with a pseudotumor and the other two groups (p<0.02 in both cases) (Fig. 1C). Overall, the group with failed implants with a pseudotumor showed lower percentages. For CD3+CD4+ cells expressing IFN-γ (considered to be primarily Tc cells expressing IFN-γ, i.e., type 1 Tc cells), overall lower percentages were also observed in the group with failed MM hip implants with a pseudotumor, and a significant difference was detected between the three experimental groups, although with p=0.049 (data not shown). Finally, the percentages of CD3+CD4+ and CD3+CD4- cells expressing IL-4 remained low, and the three groups were not significantly different (data not shown).

Discussion: Overall, this study shows significant differences in the proportions of lymphocyte subpopulations, particularly in
memory Th cells as well as in type 1 T-cells (expressing IFN-γ), in peripheral blood from patients with failed MM hip implants with a pseudotumor compared to patients with failed implants without a pseudotumor, and to patients with well-functioning implants. Specifically, patients with failed MM hip implants with a pseudotumor showed overall lower proportions of memory Th and Tc cells as well as type 1 Th cells and, to a lesser extent, type 1 Tc cells. This suggests a lower number of memory T-cells and type 1 T-cells, particularly Th cells, circulating systemically in these patients, 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 and type 1 Th cells in the tissues would be consistent with a type-IV hypersensitivity reaction.

Significance: Results suggest the presence of a type-IV hypersensitivity reaction in patients with failed MM hip implants associated with a pseudotumor. In addition, immunophenotypic differences between these patients and other MM patients 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 immunophenotypic differences, and results should be correlated to histological analyses of periprosthetic tissues.

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References: