Immunophenotypic Analysis of Peripheral Blood Lymphocytes in Patients with Hip Implant-Related Metal Hypersensitivity

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Disclosures: E.A. Lehoux: None. I. Hurda: None. S.J. Baskey: None. P.E. Beaulé: 1; Corin, Medacta. 2; MicroPort, Medacta. 3B; DePuy-Synthes, Maquet. 5; DePuy-Synthes,Corin, MicroPort. I. Catelas: 4; Baxter International Inc.. 5; MicroPort Scientific Corp..

Introduction: Current diagnostic methods of hypersensitivity reactions are not well accepted for the characterization of implant-related metal hypersensitivity, which remains a major cause for concern. A better understanding of the underlying mechanisms of hypersensitivity reactions is therefore critical, especially since it will ultimately facilitate the development of new diagnostic methods, and treatments of these reactions. Implant-related hypersensitivity reactions have been thought to be T lymphocyte cell-mediated, delayed-type IV hypersensitivity immune reactions [1]. However, Willert et al. [2] showed the presence of plasma cells, B lymphocytes, and massive fibrin exudation in tissues surrounding metal-on-metal (MM) hip implants. These observations are not characteristic of a type-IV hypersensitivity reaction [2]. Hence, the exact role of T lymphocytes and the mechanisms of hypersensitivity reactions surrounding metal implants remain unclear. While previous studies have characterized T (specifically T-helper (Th) and T-cytotoxic (Tc)), B and natural killer (NK) subpopulations in the peripheral blood of patients with a failed implant, there has been little attention to the state of lymphocyte activation (i.e., memory vs. naïve) as well as to the cytokine profile of T cells to determine the different subsets (e.g., Th1 and Th2). If a type-IV hypersensitivity reaction is prevalent, a local increase in memory T lymphocyte percentages would be expected. Furthermore, since type 1 Th cells (Th cells expressing interferon-gamma, IFN-γ) are involved in type-IV hypersensitivity reactions, a local increase in the proportion of IFN-γ-expressing Th cells would also be expected. Therefore, the objective of this study was to identify which lymphocyte subpopulations are over- or under-represented in peripheral blood of patients with a failed hip implant and a strong to predominant hypersensitivity reaction, compared to patients with a failed implant with no or a mild hypersensitivity reaction, and to healthy subjects with no implant (controls). This comparison will reveal potential differences in the systemic immune response that is expected to reflect local differences in the periprosthetic tissues. These differences will reflect the patient immunological response to metal wear and/or corrosion products and provide insight into the type(s) of hypersensitivity reaction involved in the response.

Methods: This study has been approved by our local research ethics board. Heparinized peripheral blood and periprosthetic tissue samples were obtained from consenting patients with a strong to predominant hypersensitivity reaction (3 males and 5 females with a failed MM hip implant; 60.8 ± 13.0 years old; mean time to failure of 3.5 ± 0.9 years), patients with no or a mild hypersensitivity reaction (4
males and 3 females with either a failed metal-on-polyethylene (MPE) (n=4) or MM (n=3) hip implant; 62.8 ± 14.7 years old; mean time to failure of 5.1 ± 5.9 years), and healthy subjects with no implant (controls) (15 males and 12 females; 41.1 ± 14.3 years old). The hypersensitivity reaction was characterized by histological analysis of haematoxylin and eosin (H&E) stained periprosthetic tissue samples after fixation in 10% neutral buffered formalin and embedding in paraffin wax. Levels of hypersensitivity reaction (mild to predominant) were classified based on the inflammatory infiltrate in the tissues (adapted from Campbell et al. [3]). Mononuclear cells from freshly collected peripheral blood were isolated by density gradient, and stained for surface markers of T cells (CD3, CD4 (Th) and CD8 (Tc)), B cells (CD19) and 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 subpopulations. Because of the limited number of patients in two of the groups, many of the distributions for the blood data were not normal, and therefore, 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, as well as for total memory T cells, specifically memory Th and memory Tc cells, and memory B cells (data not shown). However, significant differences between the three groups were observed for Th and Tc cells, as well as for total type 1 T cells (T cells expressing IFN-γ), and specifically type 1 Th and Tc cells (p≤0.04 in all cases). Overall, results showed larger relative proportions of Th cells and lower relative proportions of total type 1 T cells, and specifically type 1 Th and Tc cells in the group with a strong to predominant hypersensitivity reaction compared to the other two groups (p<0.05 in all cases) (Figure 1). Finally, the percentages of Th and Tc cells expressing IL-4 remained low, with no significant difference between the group with a strong to predominant hypersensitivity reaction and the other two groups (data not shown).

**Discussion:** Overall, this study shows significant differences in the proportions of lymphocyte subpopulations, particularly in type 1 T cells (expressing IFN-γ), in peripheral blood from patients with a strong to predominant hypersensitivity reaction compared to those with no or a mild hypersensitivity reaction and to the controls. These results corroborate those from Hallab et al. [4], who showed an increased lymphocyte proliferation and production of IFN-γ after in vitro cell stimulation with metal ions, suggesting the association of a type 1 Th (Th1) response with metal-induced reactivity. Specifically, results of the present study showed lower proportions of type 1 Th and type 1 Tc cells in patients with a strong to predominant hypersensitivity reaction. This suggests a lower number of type 1 T 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 hypersensitivity reaction). However, this would need to be confirmed with absolute cell counts. A local increase in the number of type 1 Th cells in the tissues would be consistent with a type-IV hypersensitivity reaction. Interestingly, all failed implants in the group with a strong to predominant hypersensitivity reaction were MM implants, with a majority (6 out
of 8) diagnosed with a pseudotumor. Increasing group sizes will allow determining if the hypersensitivity reaction is of the same nature in the presence or absence of a pseudotumor.

**Significance:** Results suggest an association between type 1 T cells and the observed hypersensitivity reaction, consistent with a type-IV reaction. In addition, the observed phenotypic differences could potentially become diagnostic markers for the detection of such a reaction. Nevertheless, group sizes need to be increased to confirm the phenotypic differences, and results should be correlated to histological analyses of periprosthetic tissues.

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ORS 2015 Annual Meeting  
**Poster No:** 1825