Titanium used in prosthetic devices can drive a potent in vivo inflammatory response and act as an adjuvant for Th2 cell differentiation

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ABSTRACT INTRODUCTION:

Prosthetic devices are composed of biomaterials that may include ceramics, titanium, cobalt, nickel, and stainless steel. Inflammation associated with implantation of prosthetic devices can become a major complication possibly contributing to osteolysis and aseptic loosening. One potential cause of such inflammation may be particulate wear debris generated from the prosthetic materials. Titanium has been considered an inert biomaterial and is used in orthopedic implants and also as a component of different dental filling materials and skin protecting cosmetics. However, some recent studies have suggested that titanium particles may contribute to inflammatory responses. In the studies described herein, we have examined whether titanium particles have inflammatory properties that can stimulate specific components of the innate immune response and act as an adjuvant to drive T helper cell development.

METHODS:

BALB/c, BALB/c IL-4−/−, BALB/c DO11.10 TCR transgenic mice, CHS/He Ouj (wild) CHS/HeJ (TLR-4−) were either purchased from The Jackson Laboratory or bred at UMDNJ animal facility. The studies reported here conformed to the principle for laboratory animal research outlined by the Animal Welfare Act and the Department of Health, Education and Welfare (National Institutes of Health) guidelines for the experimental use of animals.

DO11.10 T cell transfer

3X106 CFSE labeled DO11.10 OVA-specific CD4+ T cells were transferred to recipient BALB/c mice. Mice were immunized intraperitoneally 2 days later with either OVA peptide (30 μg) or 12.5 mg Ti particles or 4mg Alum along with 30 μg OVA peptide. After 7 days post transfer, in vivo cell cycle progression of OVA specific CD4+ T cells was assessed by measuring sequential reductions in CFSE fluorescence of KJ1-26/CD4+ cells and the proliferation index was calculated using flow cytometric analysis. OVA specific ELISPOT was developed as previously described (Liu Z et al, 2002).

Immunization and antibody measurement

Mice were immunized with OVA (50 μg) or 12.5 mg Ti particles + 50 μg OVA or 4mg Alum + 50 μg OVA and challenged with OVA alone on day 7. After 21 days of last immunization, sera were collected for measuring total and OVA specific antibody.

Analysis of PEC (peritoneal exudates cells):

Mice were immunized with PBS, Ti (12.5 μg.), Alum (8mg), and OVA (50 μg) alone or with Ti / alum. After 48 hours, peritoneal exudates cells were collected by injecting 8 ml of RPMI media. Fat associated Lymphocyte clusters (FALC) were separated and treated with collagenase-I for 1 hour at 37°C. Peritoneal fluid was collected after 24 hours of above immunization and analyzed using the Proteome Profiler kit (R&D Systems).PEC cells were blocked with Fc Block (BD Pharmingen) and then incubated with, Ly6G FITC, CD11cPE, CD11b PerCPCy5.5, SiglecF PE, FcR1 FITC, C-kit APC, DX5 PerCP, (BD Pharmingen). For characterization of Fat associated lymphoid clusters, cell suspensions were stained with Lin FITC, C-kit/APC and Sca-1 PE.

RESULTS SECTION:

We observed a potent TLR-4 independent Th2-type immune response after i.p. inoculation of a 20 micron Ti particle with OVA, compared to OVA alone, which was characterized by serum elevations in IgE and IgG1. To examine whether Ti could act as an adjuvant to drive Ag-specific Th2 cell differentiation, CFSE labeled transgenic OVA-specific DO11.10 CD4+ cells were transferred to recipient BALB/c mice and inoculated i.p. two days later with Ti plus OVA or OVA peptide or with OVA alone. Five days after inoculation, CFSE fluorescence analysis showed that donor CD4+, OVA-specific DO11.10 T cells had undergone markedly more cell cycling in recipient mice receiving Ti+OVA compared to recipient mice administered OVA alone. OVA-specific ELISPOT assays of cell suspensions from draining lymph nodes showed increases in DO11.10 T cell IL-4 expression from mice inoculated with Ti + OVA compared to mice immunized with OVA peptide alone. These studies suggest that Ti can act as an adjuvant in vivo to induce naïve T cell differentiation to Th2 cytokine producing effector T cells. To examine the early innate response that might be driving the adaptive T cell response, we assessed immune cell populations in the peritoneal cavity after i.p Ti inoculation. At 48 hours after Ti inoculation, increased infiltration of neutrophils, eosinophils and basophils were observed. Also, marked increases in both alternatively activated macrophages (M2) and the recently reported (Moro Kazuyo, 2010) Lin+ C-kit+ Sca-1 natural Th2 helper cell were observed, characteristic of Th2-type responses. This Th2-type response was not mediated by endotoxin associated with Ti particles as TLR4 KO (CHS/Hej) mice, which are unresponsive to endotoxin, showed marked increases in serum IgE and IgG1 in response to Ti + OVA. In order to characterize the initial inflammation, after 24 hours peritoneal fluids were analyzed by R&D cytokine array. Eighteen different cytokines and chemokines including IL-1β (40 folds), IL-1Ra (35 folds), IL-2 (15 folds), IL-6 (57 folds), and IL-16 (>100 folds) were increased indicating the potency of Ti as an inducer of innate inflammation.

DISCUSSION:

Our studies suggest that Ti can act as an adjuvant to drive the development of Ag-specific Th2 cells and elevations in serum IgE. This response likely occurs independently of endotoxin as it is similarly increased in TLR4-deficient mice. Ti also induces a strong innate immune response with increases in M2, basophils, and other cell populations that have been previously associated with immune responses leading to the development of Th2 cells. Further studies are required to elucidate the mechanisms by which this purportedly inert material triggers Th2-type inflammation. These findings may provide useful insights for the development of novel approaches to control inflammation associated with Ti particles.

REFERENCES: