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Treatment With Staphyloccocal Protein A Which Is Immuno-Modulatory In The Murine Collagen Arthritis Model, Does Not Increase Infection Severity In Murine Listeria Or Candida Challenge Models, In Contrast To Anti-TNF Treatment

Abstract:
#1843
Presenter:
Bernton, Edward M.D.
Co-Authors:
Lowe, Valerie BS
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Tuesday, October 29, 2013
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Exhibit Hall B2-C-D
Session Title:
Cytokines, Mediators, Cell-cell Adhesion, Cell Trafficking and Angiogenesis II
Abstract Category:
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Background/Purpose:
PRTX-100 is a highly-purified GMP staphylococcal protein A (SpA) that is currently in Phase I trials in patients with active rheumatoid arthritis (RA). SpA has diverse activities in vitro and in vivo: forming immune

complexes with IgG, SpA induces a “suppressor” phenotype in murine and human macrophages; IP or IV administration reduces disease severity in the murine CIA model. SpA can also inhibit activation of human monocyte-derived macrophages by LPS and gamma-IFN. SpA binds to Vh3 B-lymphocytes, and relocates with them to lymphoid tissues. Since anti-cytokine biologic DMARDS, in particular anti-TNF products, have been shown to increase patient susceptibility to pathogens such as listeria, fungi and TB, we compared the effects of SpA treatment to that of etanercept and anti-mouse TNF in murine models of Listeria and Candida infection.
Methods: For Listeria challenges, groups of 15 Balb/C mice were treated ip with either 10 mL/kg of 0.1% BSA, 15 mg/kg of etanercept, 50 or 250 µg/kg of SpA, or 0.2 mL of rabbit anti-mouse TNF antisera. After 4 hours mice were administered 5 x 10^10 CFU of L. monocytogenes orally. Mice were then re-treated with drugs every 48 hours x 2. Weights and mortality were recorded daily. On Days 3, 5, and 8, five mice/group were sacrificed for spleen cultures and CFU counts. For Candida challenges, groups of 15 female CD-1 mice received the same treatments. Four hours after the first treatments they were injected IV with 2 x 10^6 CFU of Candida albicans. Daily weights and mortality were recorded. On days 3, 5, and 8, five mice/group were sacrificed for kidney cultures and CFU counts.
Results: Mean values for weights, bacterial load: *Listeria challenge – BSA*: 20% mortality.10% weight loss at Day 4 was regained by Day 6. *Anti-TNF*: 21% weight loss on Day 5, 100% lethality by Day 6. *Etanercept*: 17% weight loss by Day 5, maintained through to end of study;

no mortality. Spleen bacterial counts were higher ($p<0.05$) than with BSA treatment at Day 5. SpA, 25 or 250 µg/kg; no mortality, weight loss similar to BSA-treated mice. Lower bacterial counts at Day 5 than seen following etanercept or anti-TNF treatment. *Candida challenge – BSA*: 17% weight loss by Day 5 and 10% mortality by Day 8. *Anti-TNF*: 23% weight loss on Day 5 with 100% mortality by Day 6. Kidney yeast counts higher ($p<0.05$) than BSA group on days 3 and 5. *Etanercept*: 30% weight loss by Day 7 and 60% mortality by Day 8. Mean kidney counts higher ($p<0.05$) than BSA group at days 3, 5, and 8. *SpA groups*: weight loss similar to BSA group. Kidney counts not significantly different at days 5 and 8 from BSA group; no mortality by Day 8.

Conclusion: In contrast to etanercept or anti-TNF treatment, repeated injections of SpA at 50 or 250 µg/kg did not affect disease severity or pathogen load in these challenge models with bacterial or fungal intracellular pathogens.

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Complement Activation and Anaphylatoxin Generation In Response To Staphylococcal Protein A Exposure: Ex Vivo and In Vivo Human Studies

Abstract:
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Presenter:
Bernton, Edward M.D.
Co-Authors:
Polley, Antonio ; Zondlo, Susan ; Mitchell, Lynne ; Hourcade, Dennis PhD
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Background/Purpose: PRTX-100, a highly-purified GMP staphylococcal protein A (SpA), is currently in clinical trials treating patients with active rheumatoid arthritis (RA). It has been reported that binding of SpA to rabbit Vh3 IgM antibodies could deplete complement hemolytic activity and

release C3a. Furthermore, complement activation is a postulated mechanism for dosing reactions seen in several patients after overly-rapid injection of SpA. We therefore looked at ex vivo effects of: 1. Adding SpA to pooled normal serum using a highly-sensitive modified CH50 assay; and 2. Adding SpA to fresh healthy donor blood on production of stable metabolites of C3a, C4a, and C5a, which are anaphylatoxins and known vasoactive and immunomodulatory mediators. Additionally, these analytes were measured in plasma from RA patients, before and after dosing with SpA.
Methods: *CH50 studies*: Pooled normal donor serum was incubated with various concentrations of SpA or positive controls and then assayed for residual complement activity (measured as CH50). *Whole blood studies*: Heparinized blood was incubated at 37 ℃ with 0, 250, 500, or 2000 ng/mL of SpA or with zymosan as a positive control. At 15 and 60 minute intervals, samples were evaluated using a multiplexed cytometric bead array (CBA) assay to quantify C3a, C4a, C5a stable metabolites. *Patient studies*: EDTA/Futham plasma samples were obtained before and after the first and fifth infusion with SpA, and frozen for CBA analysis of anaphylatoxins. Sequential groups of 6 patients were infused weekly with 1.5, 3.0, 6.0, or 12 µg/kg of SpA.
Results: In the CH50 study, 3 replicate experiments were performed adding serial two-fold dilutions of SpA from 4000 to 125 ng/mL. All CH50 values averaged between 85% and 108% of serum incubated without SpA, with no SpA dose response observed. CH50 values at 4000 ng/mL SpA were 114, 106, and 98% of the serum control. Incubation with a complement-activating nanoparticle reduced hemolytic activity to undetectable levels (< 5% of serum control). Using whole blood from 3 donors, and the CBA assay, C3a increased a maximum of 2.5-fold with SpA addition, compared to no addition. The mean increase with

zymosan addition was 332-fold. For C4a the maximum increase was 2.3-fold compared with a mean 99-fold for zymosan addition. For C5a the maximum increase was 2.3-fold that seen for control, compared with a mean 213-fold increase with zymosan addition. Data for pre- and post-infusion samples for 47 infusions in the first 28 patients dosed, showed 7/28 had 3-fold or greater increase in C3a (range 3 to 14 fold) but only 2/28 had a 2-fold or greater increase in C4a (range 2.4 to 2.7 fold). The mean of the ratio between pre- and post-dose values was 2.1, 1.0, and 1.1 for C3a, C4a, and C5a respectively. The maximum fold increases were 14.0, 2.7, and 3.0 respectively.
Conclusion: Ex vivo experiments do not demonstrate activation of complement in serum or whole blood by SpA at concentrations of up to 4000 ng/mL (serum) or 2000 ng/mL (blood). One quarter of patients experienced a 3-fold or greater increase in C3a after SpA treatment, which was not associated with any dosing symptoms. C4a and C5a were unaffected by treatment.