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ABSTRACTS

Oral Presentations: Friday, June 8

#1201- Therapies to Induce Tolerance Prevention and Treatment of Autoimmune Arthritis Using IL-12 Gene-Silenced Dendritic Cells

Friday, June 8
10:45 am–11:05 am

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We have recently demonstrated that dendritic cell(DC)-mediated immune modulation and deviation can be accomplished through RNA interference (RNAi), highlighting the therapeutic potential of RNAi-modified DC as antigen-specific tolerogenic vaccines. To date, an RNAi-based immune therapy for autoimmune diseases has not been reported. The current study was designed to develop siRNA-modified DC as antigen-specific, tolerogenic vaccines for prevention and intervention of autoimmune arthritis. Using small interfering RNA (siRNA) that specifically targets IL-12p35 gene (IL-12 siRNA), we have generated a type of DC that exhibits multiple tolerogenic characteristics. After inducation of RNAi in DC using siRNA, the expression of IL-12 was inhibited as determined by real time PCR and flow cytometry. IL-12 silenced DC suppressed T cell response in an MLR, and impaired Th1 differentiation *in vitro*. Immunization with IL-12 gene-silenced and type II collagen (CII)-pulsed DC (CII-pulsed/gene-silenced DC) resulted in antigen-specific non-responsiveness. Vaccination with CII-pulsed/gene-silenced DC prevented collagen-induced arthritis (CIA) onset in a murine rheumatoid arthritis model. Furthermore, administration of CII-pulsed/gene-silenced DC was sufficient to inhibit progression of CIA. The therapeutic effects were further evidenced by decreased clinical score in CIA, inhibited inflammatory infiltrates in joints, and suppressed T cell and B cell responses to CII. In conclusion, this study is the first to demonstrate the therapeutic utilization of RNAi-modified DC as antigen-specific tolerogenic vaccines for autoimmune arthritis.

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#1202- Commensal Flora and the Immune Response Helicobacter Pylori in the Pathogenesis of Chronic Autoimmune Pancreatitis

Friday, June 8
10:45 am–11:05 am

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Autoimmune chronic pancreatitis (ACP) is a type of pancreatitis characterized by a chronic inflammatory process with prominent lymphocyte infiltration leading to fibrosis of the pancreas and eventually to organ dysfunction. The cause of the disease is still unknown. Current evidence suggests an autoimmune origin for ACP, based on its frequent association with other autoimmune diseases (rheumatoid arthritis, Sjogren's syndrome, and inflammatory bowel disease) and on the presence of immunological abnormalities, including hypergammaglobulinemia, elevated serum IgG4 levels and the presence of autoantibodies, such as antibodies against carbonic anhydrase II. However, little is known about its actual pathogenesis. In order to clarify the pathogenesis of the disease, we screened a random peptide library with pooled IgG immunoglobulins derived from 20 patients with ACP.

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Among the identified peptides, one was recognized by the majority of patients' sera, but not by sera of normal donors and of patients with other autoimmune diseases. The peptide showed homology with an Helicobacter pylori derived protein and with carbonic anhydrase 5B, a protein highly expressed in pancreas, kidney and salivary glands, and with lactoferrin, considered an important autoantigen target in ACP. Anti-peptide antibodies, affinity purified from patients' sera recognize the helicobacter derived protein, carbonic anhydrase 5B and lactoferrin. Moreover antibodies against the bacterial epitope can be detected in the vast majority of patients with ACP. Our findings suggest that Helicobacter pylori infection may be linked to the pathogenesis of ACP through a molecular mimicry mechanism and that carbonic anhydrase 5B can be considered a novel autoantigen in ACP.

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OR.1 Foxp3 (Scurfy) Mutation Greatly Accelerates Diabetes in Insulin-specific TCR Transgenic Mice

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Previously we developed and described the development of a transgenic mouse expressing an insulin-specific (ins2 B:9−23) T cell receptor mouse model (BDC12-4.1). This pathogenic model exhibited spontaneous diabetes development on a backcross-1 (FVB×NOD)×NOD background. Disease development was heterogeneous and dependent on both I-Ag7 and RAG-deficient genotypes with relatively slow development of diabetes such that the median age of onset of diabetes was 49 weeks, and less than 10% of mice developed diabetes prior to 10 weeks of age. To test the hypothesis that the 12-4.1 transgenic T cells, even on a RAG−/− background, were heterogeneous with development of both regulatory and pathogenic T cells, we crossed NOD congenic BDC12-4.1 mice with C57BL/6 Foxp3 mutant mice. Expression of the single BDC12-4.1 TCR on a RAG-deficient background rescues the mouse from the scurfy phenotype at the BC1 (C57BL/6×NOD)×NOD generation. All RAG-deficient Foxp3 mutant mice (8/8) expressing the BDC12-4.1 TCR develop diabetes between 5 and 10 weeks of age with aggressive destruction of essentially all beta cells whereas prediabetic mice exhibit invasive insulitis. Disease develops equally in homozygous (I-Ag7/g7) and heterozygous (I-Ag7/b) mice significantly earlier and with greater uniformity than previously described ($P<0.0001$). Disease can be adoptively transferred from diabetic animals to NOD.scid mice. These studies provide evidence that a single transgenic TCR generates both pathogenic and Foxp3-dependent regulatory T cells and provides an experimental model to test therapies of diabetes prevention without Foxp3 dependent regulatory T cells.

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OR.2 Regulatory T Cells Require Serum for Suppression of Effector T Cell Proliferation and Express Stable Membrane-bound CD25

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CD4+CD25+ regulatory T cells (Treg) suppress effector T cell (Teff) functions and are essential for maintaining peripheral tolerance. Recent work suggests that IL-2 signaling is crucial for proper Treg development and function. In this study, we assessed the production of the soluble form of CD25 (sCD25) in human Treg or Teff cells, alone and in combination, following polyclonal activation during an *in vitro* suppression assay. We found that surface CD25 is relatively stable on Treg *in vitro*, whereas CD25 expression on isolated Teff cells is upregulated following activation; subsequently resulting in high levels of sCD25 detected in culture supernatants (mean + SD, 682.2 + 367.1 vs. 5369.5 + 2575.6 pg/ml for Treg vs. Teff cells respectively; $N=7$, $P=0.004$). The production of sCD25 can occur in an autocrine fashion and correlates with T cell proliferation ($r=0.76$, $P<0.0001$). To eliminate the influence of serum sCD25, our studies were also conducted under serum-free conditions. Surprisingly, under these conditions, Treg fail to suppress Teff cell proliferation (% suppression at a 1:1 Treg to Teff ratio; −197.8 + 270.6 for serum-free medium vs. 70.4 + 17.3 with 1.0% serum; $P=0.04$). Despite this functional defect, Treg cells still maintained their capacity to inhibit Teff cell cytokine production (e.g., IFN-γ responses were suppressed 75.6% in co-culture vs. Teff alone, $P<0.05$). These data highlight a mechanism by which Treg may obtain qualitatively greater IL-2 signals than Teff cells and suggest that serum is required for full Treg activity.

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OR.3 TGF-β-induced TCR-Tg/NOD Regulatory T Cells Suppress Both Diabetes Transfer and Islet Graft Destruction in an Antigen-dependent Manner

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