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201 The Interaction Of Bifidobacteria With Human Blood Leukocytes

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RATIONALE: Probiotics may possibly exert health benefits in enteric infections, allergic and autoimmune diseases. There is great variation in the ability of different strains of Bifidobacterium to interact with the immune cells. This study assesses the interaction of Bifidobacterium strains with human monocytes, neutrophils and lymphocytes.

METHODS: 4 Bifidobacteria strains, as well as their cell walls and lysates were assessed. Bacterial cells labeled with FITC were used for the phagocytosis assay and Reactive Oxygen Species production study by neutrophils. Blood cells were cultured with bacterial cell walls and cell lysates. Flow cytometry was used to determine cell surface markers from monocytes and lymphocytes (CD80/CD14, CD69/CD3) and ELISA to measure cytokines (TNF-a, INF-g).

RESULTS: Inter-strain variations in the ability of Bifidobacteria to be phagocytosed by neutrophils and monocytes were seen. The phagocytosis of bifidobacteria by monocytes was lower than by neutrophils, while the inter-strain variations were similar. High ability of bifidobacteria cell wall components to activate neutrophils and stimulate the generation of reactive oxygen species was noted. Significant effects of bifidobacteria cell wall components on the expression of CD69 by lymphocytes were seen. Bacterial cell walls enhanced the production of TNF-alpha and expression of CD80 by macrophages, but not INF-gamma production by lymphocytes.

CONCLUSIONS: There were significant immunomodulatory effects of the bifidobacteria strains studied as well as their isolated cell walls. These results suggest the potential use of these bifidobacteria strains in biotechnology to develop new immunomodulators or as prebiotics for treatment or prophylaxis of immunologic human diseases.

202 Worldwide Impact Of LAD2 Mast Cell Line On Mast Cell Biology Research

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RATIONALE: During culture of bone marrow-derived human mast cells, we noticed a clone of human mast cells thriving with rhSCF. This clone, named LAD2, was FcepsilonRI+/CD117+ and capable of releasing beta-hexosaminidase(20-60%), enabling study in lieu of primary mast cell cultures. To study the impact of worldwide distribution, we determined the numbers of investigators and publications resulting from LAD2 use.

METHODS: Records maintained in our lab, Technology Transfer and Intellectual Property Office, and Office of Technology Transfer were reviewed for investigator numbers and location, material transfer agreements (MTAs) and licensing agreements (LAs). Journals and their impact factors were obtained from PubMed.gov using the term LAD2. Data was displayed in Excel format.

RESULTS: As outlined in handling instructions, LAD2 cultures were maintained weekly, stored under N2 and thawed yearly for expansion and distribution to centers worldwide. Since 2001, over 300 MTAs and 50 LAs have been approved. LAD2 cells were shipped to all continents, except Antarctica. Over 80 publications in journals with impact factors 1.31 to 13.21 were documented. Intended use included degranulation by new molecules, degranulation inhibition, receptors/cell signaling and genetic marker studies. Overall investigator satisfaction was positive based on ease of obtaining cells, use/storage of cells, data duplication, and trouble-shooting of questions or problems.

CONCLUSIONS: Based on publications to date, LAD2 cells have made a substantial impact on the study of human mast cell biology. The NIAID approach via MTAs or LAs to obtain cells and have queries addressed in a timely fashion, should enable future research and become a model system for other developed and available cell lines.

203 Non c-Kit Tyrosine Kinase Expression In Mast Cell Leukemia

Dr. Joseph H. Butterfield, MD, FAAAAI; Mayo Clinic, Rochester, MN.

RATIONALE: The c-kit Asp816Val mutation is present in all subclasses of systemic mastocytosis (SM), yet the clinical courses of patients differ greatly. Treatment approaches based on inhibiting mutated c-kit have largely been ineffective, suggesting that additional tyrosine kinase genes could be overexpressed as well.

METHODS: Peripheral blood (PB) cells frozen in 1985 from a mast cell leukemia (MCL) patient whose cells were the source for the HMC-1 cell line were examined for expression of tyrosine kinase genes. After thawing and short-term cell culture, RNA was isolated from these MCL-PB cells. For control, peripheral blood cells remaining in a leukoreduction system chamber from a plateletpheresis donor were used. Microarray analysis was conducted according to manufacturer's instructions for the Affymetrix One Cycle Target Labeling and Control Reagents kit. Data was searched for increased expression of receptor and non-receptor tyrosine kinase genes. 20 receptor TK families (58 genes) and 12 non-receptor TK families (37 genes) were searched for increased expression (3^{+}fold change) compared to control leukoreduction system chamber cells.

RESULTS: The highest expression (fold change) compared to control occurred among the receptor TKs (RTKs): *v-kit* (96.9-fold change), neurotrophin tyrosine kinase receptor type 1 (NTRK1) (33.18-fold change) and Tie-1 (22.01-fold change). Non-receptor TK expression was not as marked. The highest values were for protein tyrosine kinase (PTK)2 (6.83-fold change) and PTK6 (6.61-fold change). Several RTK families were under expressed compared to control.

CONCLUSIONS: Over-expression of multiple TK genes was detected in the cells of this patient with MCL.

204 Characterization Of Systemic Mastocytosis Patients Based Solely On The Minor Criteria

Dr. Anupama Ravi, MD, Dr. Joseph H. Butterfield, MD, FAAAAI; Mayo Clinic, Rochester, MN.

RATIONALE: As part of an ongoing review of systemic mastocytosis (SM) patients diagnosed solely on the minor criteria we report preliminary findings on the occurrence of B and C findings.

METHODS: With IRB approval a retrospective chart review of SM from 1992 to 2013 is being conducted. Inclusion criteria require meeting at least 3 of the minor criteria: greater than 25% mast cells are spindle-shaped or have atypical morphology, KIT D816V mutation, aberrant CD25 expression on MC, and serum tryptase persistently >20 ng/ml. Exclusion criteria included: 1) meeting the major criterion of multifocal infiltrates of MC's (>15 MC's/infiltrate) in tryptase-stained biopsy sections of bone marrow or 2) meeting less than 3 minor criteria.

RESULTS: To date five patients fulfilled the inclusion criteria (3 males and 2 females). Three and two patients met 3 and 4 minor criteria, respectively. Four patients also had biopsy-confirmed cutaneous mastocytosis. Four patients had KIT D816V mutation. All five patients had spindle-shaped mast cells with aberrant CD25 expression. Three, three, and one patient had a serum tryptase > 20 ng/mL, 24 hour urinary 11β-prostaglandin F2α >1000ng/24 hr, and 24 hour urinary N-methyl-histamine > 200mcg/g Cr at time of referral, respectively. None of the patients revealed B findings indicative of high MC burden or C findings including cytopenias, skeletal involvement, myeloproliferation, liver impairment, or weight loss.

CONCLUSIONS: Our cohort of five SM patients solely meeting at least 3 minor criteria had no B or C findings suggesting they may be more likely to have indolent disease and better prognosis.