PARTNERSHIPS TO IMPROVE IMMUNOTOXICITY TESTING

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ABSTRACT

RESEARCH IN ITB IS FOCUSED ON THE EFFECTS THAT CHEMICALS/ENVIRONMENTAL CONTAMINANTS HAVE ON MODULATION OF THE IMMUNE SYSTEM. IMMUNE MODULATION MAY RESULT IN SUPPRESSED IMMUNE FUNCTION, WHILE EXPOSURE TO CERTAIN CONTAMINANTS MAY RESULT IN HYPERSENSITIVITY REACTIONS (E.G., ASTHMA OR CUTANEOUS INFLAMMATION). IN ORDER FOR THE OFFICE OF PREVENTION, PESTICIDES & TOXIC SUBSTANCES TO IDENTIFY AND DETERMINE THE POTENTIAL OF CHEMICALS, REGULATED UNDER THE TOXIC SUBSTANCES CONTROL ACT AND THE FEDERAL INSECTICIDE, FUNGICIDE & RODENTICIDE ACT, TO SUPPRESS OR INDUCE IMMUNE FUNCTION, THE DEVELOPMENT AND VALIDATION OF SENSITIVE AND PREDICTIVE IMMUNE SYSTEM ASSAYS IS CRITICAL. ITB HAS AND IS INVOLVED IN COLLABORATIONS WITH GOVERNMENT, INDUSTRY AND ACADEMIC LABORATORIES, ADDRESSING ISSUES RELATED TO THE SENSITIVITY OF THE IMMUNE ENDPOINTS USED TO DETECT IMMUNOSUPPRESSION AND HYPERSENSITIVITY. PARTNERSHIPS BETWEEN ITB AND OTHER LABORATORIES INCLUDE THE FOLLOWING:

A. EVALUATION OF CYTOKINE mRNA PROFILES FOR THE POTENTIAL TO AID IN IDENTIFICATION OF AIRWAY SENSITIZERS WITH TWO INDUSTRY LABS. THE DATA DEMONSTRATED DIVERSE RESPONSES OF DIFFERENT LOW-MOLECULAR-WEIGHT CHEMICALS, WHICH SUGGEST THE NEED TO DEFINITIVELY LINK THESE DIFFERENCES IN IMMUNE RESPONSES TO DIFFERENCES IN RESPIRATORY RESPONSES.

B. PARTICIPATED WITH 8 ACADEMIC AND GOVERNMENT LABS, AND 6 INDUSTRY LABS, IN AN INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY STUDY IN RATS, COMPARING THE SENSITIVITY OF IMMUNE FUNCTION ASSAYS VERSUS HISTOPATHOLOGY FOLLOWING IMMUNOSUPPRESSION BY AZATHIOPRINE OR CYCLOSPORIN. THE ANTIBODY RESPONSE AND THE PERFORMANCE OF "ENHANCED PATHOLOGY" EACH APPEARED TO IDENTIFY IMMUNO-SUPPRESSION AT RELATIVELY COMPARABLE DOSES.

C. THE EVALUATION AND IDENTIFICATION OF THE SENSITIVITY OF TWO ANTIBODY RESPONSE ASSAYS, FOR ROUTINE IMMUNOTOXICITY TESTING, WHICH INVOLVED 2 INDUSTRY LABS AND 1 ACADEMIC LAB. THE RESULTS REVEALED THAT THE ANTIBODY PRODUCING PLAQUE FORMING CELLS (PFC) ASSAY WAS MORE SENSITIVE THAN SERUM ANTIBODY TITERS.

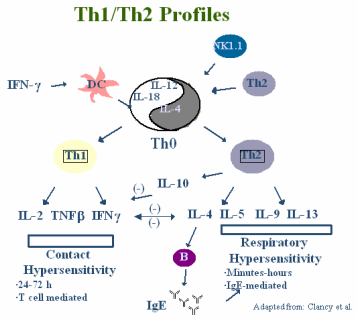
D. PARTICIPATED IN A SERIES OF STUDIES IN CONJUNCTION WITH THE NATIONAL TOXICOLOGY PROGRAM, NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES AND THE NHEERL NEUROTOXICOLOGY DIVISION, FOCUSING ON EXAMINATION OF THE EFFECTS THAT PESTICIDES HAVE ON THE DEVELOPING RATS' REPRODUCTIVE, IMMUNE AND CENTRAL NERVOUS SYSTEMS. PERINATAL EXPOSURE TO HEPTACHLOR AND METHOXYCHLOR RESULTED IN IMMUNOSUPPRESSION IN THE ADULT RATS.

E. PARTICIPATED IN COLLABORATIONS WITH THE INTERNATIONAL LIFE SCIENCES INSTITUTE IN ORGANIZING IMMUNOTOXICOLOGY WORKSHOPS ON RESPIRATORY HYPERSENSITIVITY AND DEVELOPMENTAL IMMUNOTOXICITY, AND A POTENTIAL WORKSHOP ON ALLERGENICITY OF BIOTECHNOLOGY PRODUCTS.

F. WE ARE PRESENTLY SUPPORTED BY THE AMERICAN CHEMISTRY COUNCIL TO PERFORM STUDIES TO DETERMINE THE SUSCEPTIBILITY OF THE DEVELOPING IMMUNE SYSTEM TO KNOWN IMMUNOSUPPRESSANTS.

G. WE ARE ORGANIZING A WORKSHOP ON PRACTICAL GENOMICS AND IMMUNOTOXICITY FOR SEPTEMBER. (THIS POSTER DOES NOT REFLECT EPA POLICY).

A.) EVALUATION OF CYTOKINE mRNA PROFILES FOR IDENTIFYING AIRWAY SENSITIZERS USING THE RIBONUCLEASE PROTECTION ASSAY (RPA). AIRWAY HYPERSENSITIVITY IS T-helper-2 (Th_2) CELL ASSOCIATED, WHILE CONTACT HYPERSENSITIVITY IS T helper-1 (Th_1) CELL ASSOCIATED. DISTINCT CYTOKINE PROFILES PRODUCED BY THESE DIFFERENT CELLS MAY PROVIDE A MEANS OF DISTINGUISHING RESPIRATORY SENSITIZERS FROM CONTACT SENSITIZERS (2 industry labs & ITB).



#1. MICE WERE EXPOSED TO TRIMELLITIC ANHYDRIDE (TMA) OR DINITROCHLOROBENZENE (DNCB) ON THEIR SHAVED FLANKS AND EARS. TOTAL mRNA WAS EXTRACTED FROM EAR DRAINING LYMPH NODE CELLS. CYTOKINES (i.e, IL4, IL10, AND IL13) WERE INCREASED IN RESPONSE TO TMA vs DNCB. THE DATA SUGGEST THAT QUANTITATIVE DIFFERENCES IN Th_2 RESPONSES BETWEEN TMA AND DNCB MAY BE DEMONSTRATED OVER A WIDE RANGE OF DOSES AND THAT THESE DIFFERENCES MAY BE DETECTED BY RPA FOLLOWING DERMAL EXPOSURE. IMPACT: RPA MAY BE A MORE SUITABLE ALTERNATIVE TO ENZYME-LINKED IMMUNO-SORBENT ASSAY (ELISA) FOR ASSESSING CYTOKINE PROFILES.

(Plitnick, L.M., Loveless, S.E., Ladics, G.S., Holsapple, M., Selgrade, M.J., Sailstad, D.M., Smialowicz, R.J., Toxicol.Appl. Pharmacol. 179: 145-154, 2002.)

#2. FOUR KNOWN RESPIRATORY ANHYDRIDES WITH KNOWN RESPIRATORY SENSITIZATION POTENTIAL (i.e., TRIMELLITIC ANHYDRIDE (TMA), MALEIC ANHYDRIDE (MA), PHTHALIC ANHYDRIDE (PA) AND HEXAHYDROPHTHALIC ANHYDRIDE (HHPA) WERE APPLIED TO THE SHAVED FLANKS AND EARS OF MICE. SIGNIFICANT INCREASES IN Th_2 vs Th_1 CYTOKINE mRNA WERE OBSERVRD FOR ALL THE ANHYDRIDES TESTED. IMPACT: RESULTS SUGGEST THAT RPA HAS PONTENTIAL TO SERVE AS A SCREEN FOR DETECTION OF LOW MOLECULAR WEIGHT AIRWAY SENSITIZING CHEMICALS.

(Plitnick, L.M., Loveless, S.E., Ladics, G.S., Holsapple, M., Smialowicz, R.J., Woolhiser, M.R., Anderson, P.K., Smith, C., Selgrade, M.J., Toxicology 193:191-201, 2003.)

#3. ISOCYANATES ARE LOW MOLECULAR WEIGHT CHEMICALS WHICH ARE IMPLICATED IN ALLERGIC ASTHMATIC-TYPE REACTIONS. A COMPARISON OF SIX ISOCYANATES (i.e., TOLUENE DIISOCYANATE (TDI), DIPHENYL-METHANE-4,4'-DIISOCYANATE (MDI), DICYCLOHEXYLMETHANE-4,-DIISOCYANATE (HMDI), ISOPHORONEDIISO-CYANATE (IPDI), p-TOLYL(MONO)ISOCYANATE (TMI), AND META-TETRAMETHYLENE XYLENE DISSOCYANATE (TMXDI)). CYTOKINE mRNA PROFILES WERE COMPARED AMONG THE SIX ISOCYANATES INDICATING THAT ALL SIX WERE CHARACTERISTIC OF A Th_2 RESPONSE.
IMPACT: WHETHER THESE Th_2 CYTOKINE RESPONSES ARE PREDICTIVE OF THE ASTHMAGENS EMPLOYED IN THIS STUDY REMAINS TO BE DETERMINED.

(Plitnick, L.M, Loveless, S.E., Ladics, G.S. , Holsapple, M.P., Smialowicz, R.J., Woolhiser, M.R., Anderson, P.K. , Smith, C., Selgrade, M.J., Toxicology 207:487-499, 2005.)

B.) PARTICIPATION IN AN INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY STUDY COMPARING THE SENSITIVITY OF IMMUNE FUNCTION ASSAYS VERSUS HISTOPATHOLOGY, FOLLOWING IMMUNOSUPPRESSION 0F RATS BY AZATHIOPRINE (AZA) OR CYCLOSPORIN (CYA) EXPOSURE (8 ACADEMIC, 7 GOVERNMENT, 6 INDUSTRY LABS, AND ITB).
DECREASED BODY WEIGHTS WERE OBSERVED IN A MINORITY OF LABS AT THE HIGHEST DOSE OF AZA AND CYA. DOSE-RELATED DECREASES IN PERIPHERAL BLOOD TOTAL LEUCOCYTE COUNTS, PARTICULARLY LYMPHOCYTES, WERE OBSERVED. HISTOPATHOLOGY REVEALED OVERALL REDUCED CELLULARITY IN THE THYMUS, SPLEEN, PEYER'S PATCHES, AND LYMPH NODES. AZA TREATED RATS HAD MINIMAL ALTERATIONS IN THE ANTIBODY RESPONSE (i.e., PLAQUE FORMING CELL, PFC) TO SHEEP RED BLOOD CELLS (SRBCs). HOWEVER, CYA EXPOSED RATS HAD MORE PRONOUNCED DECREASED SRBC ANTIBODY RESPONSES. A DECREASE WAS OBSERVED IN THE LYMPHOPROLIFERATIVE RESPONSE TO THE MITOGEN CONCANAVALIN A (ConA) AT THE HIGHEST DOSE OF CYA. IMPACT: PATHOLOGY INVESTIGATIONS SPECIFIED IN GUIDELINE 407 (OECD,1981) DID NOT REVEAL IMMUNE SYSTEM EFFECTS. HOWEVER, ADDITIONAL"ENHANCED" PATHOLOGY INVESTIGATIONS DID.

(Dayan,A.D., Kuper, K., Madsen, C., Smialowicz, R.J., Smith, E., Van Loveren, H., Vos, J.G., and White, K./L., Toxicology 125:183-201, 1998.)

C.) COMPARISON OF THE SENSITIVITY OF TWO ANTIBODY RESPONSE ASSAYS, FOLLOWING EXPOSURE TO CYCLOPHOSPHAMIDE (CP) OR DEXAMETHASONE (DEX) REVEALED THAT THE PLAQUE FORMING CELL (PFC) ASSAY WAS MORE SENSITIVE THAN THE ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) (1 ACADEMIC, 2 INDUSTRY LABS AND ITB).

LOWEST DOSE OF EITHER CP OR DEX AT WHICH STATISTICAL SIGNIFICANCE WAS OBSERVED

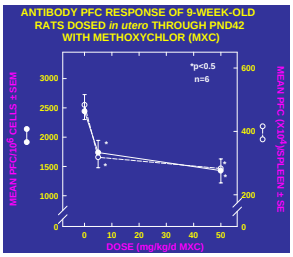
	LAB 1		LAB 2		LAB 3		LAB 4	
	CP	DEX	CP	DEX	CP	DEX	CP	DEX
RAT PFC	1*	1	10	1	10	0.1	10	1
RAT ELISA	30	NS**	10	5	10	3	10	5
MOUSE PFC	5	3	5	1	0	NS	20	NS
MOUSE ELISA	10	NS	10	3	5	NS	20	NS

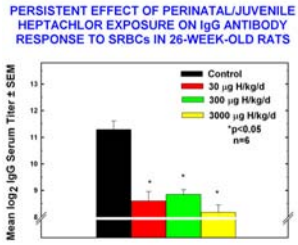
*mg/kg, ** not significant at any dose

IMPACT: THE PFC ASSAY APPEARS TO BE MORE SENSITIVE THAN THE ELISA.

(Loveless, S. E., Ladics, G. S., Smith, C., Holsapple, M.P, Woolhiser, M. R., Anderson, P.K., White, K.L. Jr., Musgrove,D.L., Smialowicz, R.J., and Williams,W.C., In preparation).

D.) COLLABORATIONS WITH THE NATIONAL TOXICOLOGY PROGRAM (NIEHS) AND NEURO-TOXICOLOGY DIVISION (NHEERL) ON PESTICIDE EFFECTS ON THE DEVELOPING RATS' REPRODUCTIVE, IMMUNE AND CENTRAL NERVOUS SYSTEMS. PERINATAL EXPOSURE TO HEPTACHLOR AND METHOXYCHLOR RESULTED IN PERSISTENT IMMUNOSUPPRESSION.





IMPACT: RESULTS SUGGEST A LONG LIVED SUPPRESSION OF THE ANTIBODY RESPONSE TO SRBCs IN RATS EXPOSED PERINATALLY TO THE PESTICIDES METHOXYCHLOR OR HEPTACHLOR.

(Chapin, R.E., Harris, M.W., Davis, B.J., Ward, S.M., Wilson, R.E., Mauney, M.A., Lockhart, A.C., Smialowicz, R.J., Moser, V.C., Barone, S., Padilla, S., Burka, L.T., Collins, B.J., Fundam. Appl. Toxicol. 40:138-157,1997.)

(Smialowicz, R.J., Williams, W.C., Copeland, C. B., Harris, M.W. , Overstreet, D., Davis, B.J., Chapin,R.E., Toxicol. Sci. 61:164-175, 2001.)

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