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Shigelosis: A new approach for understanding the mechanism

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In the last 12 months we have focused on two main issues regarding the interaction between Shiga toxin and mast cell/basophilic cells: 1. Development of a system for enrichment of human basophils from peripheral blood leukocytes; 2. The effect of dexamethasone on mast cell Shigela toxin interaction.

First, I would like to summarize briefly Dr. Cruz's visit to my lab. The two weeks' visit was very fruitful. The three main aims of his visit were:

1. To become familiar with the mast cell field.
2. To be introduced to the scientists in Israel who are working on Shigelosis.
3. To discuss the status of our collaborative program.

Dr. Cruz followed very closely the procedures of cultivating both mouse and human cultured mast cells and the various ways of activating these types of cells, including the degranulation assays and the measurement of the various mediators released from these cells. In parallel, almost every day we

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spent a few hours in discussion and lectures. During his stay in Israel we spent one day of discussion with scientists from Tel Aviv University who are working on a few aspects of Shigelosis.

Dr. Cruz collected stools from children suffering from Shigelosis and extracted the leukotrienes from these stools. Now in Israel we are analyzing the amount of LTC<sub>4</sub> in each extract. We will find out whether there is correlation between the amount of LTC<sub>4</sub> which will be detected, and the disease state.

#### Interaction between human basophils and Shigela toxin

One of the approaches which we are trying now to address is the possible interaction between human peripheral blood basophils and Shigela toxin. Therefore, an effort was made to purify the basophils which are 0.1% of the leukocytes, from the total leukocytes. Using techniques such as density gradients, adhesion and elutriation, we achieved up to 90% purity of basophils under sterile conditions, whereas the yield was usually 50%. However, human peripheral blood basophils (HPBB) are very short living cells and, moreover, they tend to perform irreversible aggregates within a short period of time. A comparison analysis was performed between the ability of 9 mouse embryonic skin fibroblast monolayer and 9 3T3 fibroblast monolayer to induct HPBB viability for a long period of time, and to prevent aggregation. It was found that the mouse embryonic skin fibroblast monolayer, in contrast to 3T3 fibro-

blast monolayer, was able to keep the HPBB viable for over two weeks and prevent them from making aggregates. This of course has tremendous implications for pharmaceutical studies on HPBB in general. In particular, this development gives us the opportunity to start working on HPBB instead of working on similar cells which are developed and differentiated under in vitro conditions.

We are now trying to determine whether Shiga toxin has any long term effects on these cells.

#### The effect of dexamethasone on mast cell-Shiga toxin interaction

Abl transformed mouse fetal liver derived mast cells were treated for 72 hours with various doses of dexamethasone. Then the cells were added to a 96 well microtiter plate at a density of  $1 \times 10^5$  cells/well/0.2 ml. Various doses of Shiga toxin - 10 to 200  $\mu\text{g}$  - were added to replicate cells. The cells were labelled with 0.5  $\mu\text{Ci}$   $^3\text{H}$ -thymidine for 4 h at  $37^\circ\text{C}$ , transferred on to glass fiber filter paper, water lysed and washed in an automated cell harvester unit. The incorporation of radioactivity into cell DNA was

quantitated. The following results were obtained:

**<sup>3</sup>H-THYMIDINE INCORPORATION**

Shiga toxin $\mu\text{g}/10^6$ cells	Dexamethasone (M)	c.p.m. mean $\pm$ S.E.
0	0	2650 $\pm$ 150
10	"	2318 $\pm$ 300
20	"	2130 $\pm$ 120
50	"	1994 $\pm$ 212
100	"	1899 $\pm$ 75
200	"	1760 $\pm$ 125
0	$10^{-7}$	511 $\pm$ 25
10	"	332 $\pm$ 11
20	"	337 $\pm$ 25
50	"	436 $\pm$ 15
100	"	321 $\pm$ 33
200	"	382 $\pm$ 25
0	$10^{-8}$	754 $\pm$ 55
10	"	491 $\pm$ 60
20	"	508 $\pm$ 21
50	"	434 $\pm$ 49
100	"	352 $\pm$ 29
200	"	530 $\pm$ 26

These results indicate that Shiga toxin induce mast cell sensitivity to steroids which may have some potential therapeutical implications. The mechanism will be elucidated in the future.