Project Progress Summary

Section 1: PROJECT IDENTIFICATION Information to be provided for project identification	**NOT CONFIDENTIAL**

Title of the project: A new technology for fluorescent „cell chip" immunotoxicity testing

Acronym of the project: IMMUNOTOX CELL CHIP

Type of contract: Shared-cost RTD action		**Total project cost** 1,474,059 €
Contract number **QLK4-CT-2000-00787**	**Duration** 36 Months	**EU contribution** 1,007,968 €
Commencement date 1 January 2001	**Period covered by the progress report** 1 January 2001 – 31 December 2003	

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Key words

Immunotoxicity, In vitro, Cell lines, Cytokine expression, Reporter gene

World wide web address

http://www.immunotox.pl
http://bio.iimcb.gov.pl/chip/chip.htm

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Section 2: Project Progress Report **NOT CONFIDENTIAL**

Objectives:

Every year there are new chemicals introduced into the occupational and environmental settings, which together with those already present may increase the risk of different adverse health effects. Epidemiological data clearly shows increase in prevalence of immunological disorders, which in part can be related to the xenobiotic exposure. Immunotoxicity testing is difficult and it is rather generally accepted that it cannot be accomplished with a single test. The difficulties related to predictive testing for immunotoxicity are in part related to multiple molecular and cellular targets of immunotoxicants actions that have to be taken into account.

The main objective of this project is development of a new system for testing immunotoxic effects of xenobiotics *in vitro*, which would not only indicate perturbation of immune system but would also allow elucidating the potential mechanism of immunomodulation. Our approach utilizes the knowledge about pleiotropic activities of cytokines that are critical regulators orchestrating the immune response by interconnecting dispersed elements of immune system into one functional entity. The new system will not involve experimental animals but instead will be based on a number of immortalized mammalian cell lines representing different phenotypes of cells regulating immune response *in vivo*. These cell lines will be tested in a uniform high throughput system for expression of a number of cytokine genes. To this end specialized reporter cell lines will be generated and used to detect signals, which upregulate and downregulate expression of immunomodulatory cytokines upon contact of these cells with tested xenobiotic. The entire panel of reporter cell lines will be then pre-validated as a tool for testing immunotoxicity using data derived from already established tests as a reference. Then, it will be proposed, how to incorporate cell chip into a battery of screening tests for immunotoxicity, and how this system might generate information complementary to information obtained with other tests.

Results and Milestones:

We have developed a new system for *in vitro* immunotoxicity testing, which employs changes in cytokine expression observed *in vitro* as an endpoint indicating potential for perturbation of the immune system *in vivo*. To this end a 30 different of DNA constructs designed in such a way that expression of reporter fluorescence protein depends of regulatory sequences derived from different cytokine genes were generated. These DNA construct including reporter vectors for *IL-2*, *IFN-γ, IL-4*, *IL-1β*, *TNF-α, and β*–actin were employed for genetic modification of cell lines and resultede in development of a large collection of reporter cell lines. Upregulation of EGFP-mediated fluorescence upon stimulation with cell specific stimuli was observed in multiple reporter cell lines derived from lymphocytes, mast cells, keratinocytes and macrophages. Furthermore, this upregulation of EGFP expression was paralleled with upregulation of endogenous cytokine expression. Morphological and functional features of selected cell lines expressing EGFP under the control of cytokine promoters were compared with maternal cell lines and this comparison showed that critical functional features of the maternal cell lines were preserved in EGFP expressing cells (Fig. 2). The prototype of "Fluorescent Cell Chip" (FCC) was assembled based on selected reporter cell line and tested. In testing of the prototype of FCC chemicals with known immunotoxic activities mediated compound-specific pattern of inhibition and activation of reporter gene expression. Thus the prototype of the "Fluorescent Cell Chip" has demonstrated potential for application as a predictive screening test for immunomodulatory activities of chemicals. The major advantage of this approach is the possibility to apply this test in high throughput screening of high number of compounds for their well defined biological activity. The usefulness of this approach for practical testing will be revealed following further analysis of multiple chemical compounds of known activity *in vivo*.

Benefits and Beneficiaries:

The new chemical safety regulation being now introduced require more than 30 000 chemicals to be tested to different extend for possible toxicity. Even by the modest assessment this testing will be accomplished not earlier than in 2048. A ban for the use of laboratory animals in the safety evaluation of cosmetics has just been introduced. Therefore the proper validated alternative tests are becoming more and more important as a way to protect consumers from risk of exposure to harmful chemicals. Therefore there is an increasing demand, for methods suitable for high-throughput screening that increase the speed and reduce the cost per chemical entity for safety evaluation. Technology developed by the "Immunotox Cell Chip" project has demonstrated potential for application as an alternative test to be applied in high throughput screening of high number of chemicals.

Future Actions (if applicable):

Fluorescent Cell Chip is now at the stage of the prototype. The usefulness of this technology will be revealed following further analysis of multiple chemical compounds of known activity *in vivo*. Furthermore, to become a practical testing device this test has to be validated. Work on validation and implementation of the Fluorescent Cell Chip is already underway. Additional commercial partners are sought to further develop, validate, and implement this test to practice..