The Germinal Centre Reaction - Genetic and proteomic analysis of factors important for survival and growth of B lymphocytes

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Avhandlingen baseras på följande delarbeten:

I. Zander L, Bemark M, Immortalized mouse cell lines that lack a functional *Rev3* gene are hypersensitive to UV irradiation and cisplatin treatment. DNA Repair (Amst). 2004 Jul 2; 3(7):743-52

II. Zander L, Bemark M, Identification of genes deregulated during serum-free medium adaptation of a Burkitt's lymphoma cell line. Cell Proliferation. 2008; 41:136-55

III. Zander L, Friskopp L, Bäckström M, Bemark M, Proteomic analysis of proteins secreted by germinal centre B lymphocytes. Manuscript

IV. Zander L, Bemark M,Spontaneous loss of MHC class II expression in a transformed B cell line – a potentially new mutation leading to Bare lymphocyte syndrome. Manuscript

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During an immune response the B lymphocytes main function is to produce antibodies in the specific defence against the pathogen. When naïve B lymphocytes become activated by binding of an antigen, the cells differentiate to become antibody producing plasma cells. During this process, some cells form germinal centres after interaction with T lymphocytes, where the immunoglobuline (Ig) genes are differentiated, to evolve high affinity plasma cells or memory B lymphocytes.

We have used the Burkitt lymphoma cell line Ramos to study the germinal centre reaction. Ramos cells are normally cultured in medium containing serum, but we have adapted Ramos cells to long-term survival in a serum-free medium. The serum-free cells are more sensitive to dilution, which indicates a production of autocrine growth factors. We have studied the gene expression changes that occur during adaption to serum-free media by global gene expression analysis, and we found several deregulated genes involved in cell-cycle regulation and apoptosis. We also identified a Ramos cell line deficient in MHC class II expression, resembling the situation during Bare lymphocyte syndrome. The cause of this deficiency is studied by examining the function of transcription factors regulating MHC class II expression.

Germinal centre B lymphocytes are highly susceptible to apoptosis unless rescued by survival signals from T lymphocytes and follicular dendritic cells. In an effort to study these interactions we have isolated and identified secreted extracellular proteins produced by serum-free Ramos cells. The expressions of these proteins were also examined in tonsil germinal centre B lymphocytes and the levels were compared with cells in the pre- and post-germinal centre stage of the tonsils.

During the germinal centre reaction the antibody gene of B lymphocytes are differentiated through somatic hypermutation and class switch recombination. These events are dependent on the AID mediated cytidine deamination and involve different DNA repair systems, many of which involve error-prone polymerases. We have studied the function of one of these, Polymerase ζ, by establishing mouse fibroblast cell lines deficient of the Rev3 subunit of Pol ζ. Rev 3 deficient cells are more sensitive to cell cycle arrest caused by UV-radiation or cisplatin treatment than cells with a functional Pol ζ, confirming a function of Pol ζ in the translesion synthesis over DNA nicks and crosslinking lesions.

Key words: B lymphocyte, germinal centre, transformation, serum-free, MHC class II, Bare lymphocyte syndrome, extracellular proteins, DNA repair, Polymerase ζ

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