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Dextran Sulphate inhibits Cell Adhesion in Tissue Culture

L CELLS may be grown in defined medium either in monolayer culture or as suspension cultures in spinner flasks. The adaptation of these cells from one growth behaviour to the other, however, requires the time consuming selection of proper mutants and/or changes in the composition of their medium (Ca^{2+}, Mg^{2+} and serum concentration) probably causing further selection among the population.

I have circumvented these difficulties by adding the polyvalent anion dextran sulphate (molecular weight (M_w) 5×10^5, Pharmacia Fine Chemicals, Sweden) to the medium (BME supplemented with 5% foetal bovine serum) of L cells (L-929, American type tissue collection, NIH) growing in monolayers on either glass or plastic supports. Dextran sulphate is added at a concentration of 1 mg per 4×10^7 cells per 100 ml medium. 5 to 10 min after the addition, approximately 90% of the cells are found suspended as single cells in the medium, from where they can be either collected for further experiments (cell counts, colony forming assays, isotope labelling) or transferred to spinner cultures.

The spinner culture holds 200 ml, which is seeded with 4×10^7 cells. After a lag of 3 to 4 h, the cells enter exponential growth. Maximum cell concentration reached in BME+5% foetal bovine serum+10 μg dextran sulphate ml^{-1} is 10^6 cells ml^{-1}.

Spinner cultures are easily transformed into monolayers by simple dilution of the medium with nine parts of BME+5% foetal bovine serum without dextran sulphate.

The growth characteristics of both spinner cultures and monolayers manipulated in this way are identical to the original L cells[1] in respect of generation time (22 h$\pm$2.2 h); t_{G1} (8 h); t_S (10 h); t_{G2} (4 h); and t_M (1 h). The colony forming ability in soft agar (20 to 30%, depending on the serum batch) and the response to cytostatics are also identical to the original L cells. The c.p.m. per cell in labelling experiments involving DNA, RNA or proteins is generally increased in suspension cultures over that of monolayers, probably due to facilitated uptake of labelled precursors as a result of larger contact area.

Thus, I have used single-cell suspensions with dextran sulphate to establish perfect monolayer cultures in roller tubes (1,900 cm²) suitable for synchronisation by mechanical selection of mitotic cells[2]. Further results pertinent to this part of my work will be published elsewhere.

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Reactivity of Lymphoid Cells from Mice Humorally Tolerant to Histocompatibility Antigens

WE have solubilised and partially purified the H-2^a histocompatibility antigen of the mouse. All of the detectable H-2^a serological specificities have been preserved[1] including those occurring in a single allelic form: four at the *D* locus and twenty-three at the *K* locus. The immunogenic character[1] as revealed in second set skin and embryonic heart rejection and specific immunological enhancing capacity[2] against two strain A tumours remained intact. Thus it was presumed that our soluble antigen, occurring in a single narrow peak (F2 fraction) following G-150 Sephadex column fractionation, represented a complete molecule since the *H-2^d* and *H-2^k* loci containing the determinants detected are located at opposite ends of the H-2 complex. This F2 fraction was capable of inducing humoral tolerance but not, however, cell-mediated tolerance[3] in congenic B10.D2 mice treated with daily injections of the F2 fraction from birth. The B10.D2 mice made tolerant to this material were unable to produce cytotoxic, haemagglutinating or enhancing antibodies but nonetheless effectively rejected B10.A skin grafts. Presumably this antigenic material made tolerant the bone marrow derived B lymphocytes.

Recent evidence indicates that the major histocompatibility complex (MHC) of the mouse consists of different regions, some of which are more important than others in the different immune phenomena. For example, the Ir region of the H-2 complex, closer to the *H-2^k* locus, seems to control immune responses to certain synthetic polypeptides[4] and to IgA and IgG immunoglobulins[5,6]. This region is also thought to control graft-*versus*-host (GvH) activity and mixed leukocyte culture (MLC) reactions but not to be significantly involved in skin graft rejection or in cell-mediated lysis (CML). Different classes (subsets) of T cells seem to be involved in the different reactions[7]. The *K* and *D* loci seem to control serologically detectable specificities that are also responsible for skin graft rejection. We have investigated in our humorally tolerant mice those T-cell mediated immune functions such as the GvH and MLC reactions and CML that are evidently governed by gene products or T-cell populations differing from those governing skin graft rejection and SD reactions.

B10.D2 mice less than 18 h old were inoculated intravenously with 5–10$\times10^6$ washed spleen cells from congenic B10.A or (B10.A$\times$B10.D2)F_1 mice. Approximately 25% of these mice have retained B10.A skin grafts for more than 150 d. B10.D2 mice were also inoculated intravenously at birth and with continuing daily intraperitoneal injections for 30 d with 40 μg of our soluble H-2^a antigen. (The H-2^a antigen used here had approximately the same specific activity against antisera

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