Inhibition and Stimulation by 5-Bromodeoxyuridine of Erythropoiesis by Chick Blood Island Cells

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Summary. Erythropoiesis in liquid cultures of cell populations resolved from chick blastodiscs at the primitive streak and head-fold stages was totally inhibited by 5–8 μg/ml of 5-bromodeoxyuridine. However, concentrations of 0.2 μg/ml of the nucleoside enhanced the numebr of erythroid cells formed.

We[2] have recently developed procedures for resolution of cell populations from single-cell suspensions of primitive streak and head-fold chick blastodiscs (ca. 18–20 h incubation[3]). Two distinct populations which form foci of erythropoiesis readily visible to the naked eye when incubated as cell reaggregates were isolated. They differed in sensitivity to inhibition of subsequent hemoglobin formation following brief exposure to a high concentration of 5-bromodeoxyuridine (5BdU)[2]. Further, the more sensitive population (EP1) formed foci of erythropoiesis containing all the elements of organized blood islands, whereas the other population (EP2) yielded only loose assemblies of free erythroid cells[4]. It therefore seemed possible[4] that progression of cells from population EP1 into population EP2 could account for the increased resistance to inhibition of hemoglobin formation by 5BdU observed with the intact chick blastodisc as it develops beyond the stage of the primitive streak[5–7].

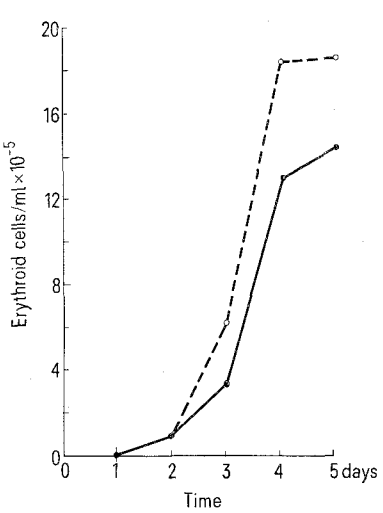
We have therefore examined the sensitivities of cells of the 2 populations to inhibition of hemoglobin formation by 5BdU in liquid culture. No difference in sensitivity was found. However, we did observe a stimulation of erythropoiesis in cultures of both cell populations in the presence of very low concentrations of 5BdU.

Materials and methods. Blastodiscs of the Shaver Starcross No. 288 line of White Leghorn fowl were explanted onto a solid minimal medium[8] and those at the primitive streak and head-fold stages of development were selected. They were detached from their supporting vitelline membranes, dispersed as single-cell suspensions and resolved into the 2 erythropoietic cell populations as previously described[2]. Each population of cells was resuspended at a concentration of 1–2 × 10^7 cells/ml in the medium previously used for studies of dispersed cell preparations in liquid culture[9], which had been pre-warmed. The suspensions were distributed in 0.8 ml volumes in a series of 35 × 10 mm tissue culture dishes (Falcon plastics No. 3001) and to each was added a further 0.2 ml of medium containing any additions. Each dish was incubated in an individual moist chamber as previously described[9], except that for these cultures an atmosphere of air-5% CO_2 was required for optimal erythropoiesis. Samples of the cells in suspension were withdrawn at intervals and the concentrations of erythroid cells were determined as previously described[9].

Results and discussion. We observed no clear difference between the 2 resolved cell populations in sensitivity to inhibition of erythropoiesis by 5BdU (Table). Erythropoiesis was totally inhibited by 5BdU at 5 or 8 μg/ml, and marked inhibition was observed at concentrations of the analogue as low as 1 μg/ml. There was some variation between different experiments in the apparent degree of inhibition caused at the lower drug concentrations, which we believe reflects variation in the amount of residual yolk retained by the cells.

Experiment No.	Type of population	Erythroid cells × 10^{-5}/ml at BdU μg/ml				
		0	0.5	1	2.5	8
1	EP1	24.2	27.3	17.9	3.3	0
2	EP1	12.6	10.8	6.0	2.1	0
3	EP2	12.2	9.5	4.4	0.1	0
4	EP2	10.5	11.3	7.6	2.3	0

Maximum count at 4 days.



Stimulation of erythropoiesis by 5-bromodeoxyuridine. Erythropoietic population EP2 was resolved from dispersed cell suspensions of chick head-fold blastodiscs by sedimentation in Ficoll density gradients and resuspended in warm medium without (● —●) and with (○ — ○) 0.2 μg/ml of 5-bromodeoxyuridine. Samples of the cultures were taken at intervals and the concentration of chick erythroid cells determined.

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Even lower concentrations of 5BdU appeared to cause a slight increase in numbers of erythroid cells formed in some experiments (e.g. Table, experiments 1 and 4). Further examination of this possibility established that maximum numbers of erythroid cells were consistently increased by about 40% in cultures of either cell population containing 0.2 μg/ml of 5BdU. Data for a representative experiment with cells of population EP2 are given in the Figure. Only slight and inconsistent stimulation was obtained at concentrations of 5BdU of 0.1 and 0.5 μg/ml, or in cultures of mechanically dispersed blastodiscs[9] at 0.2 μg/ml.

5BdU has been previously reported to suppress erythropoiesis by cells of the primitive streak chick blastodisc, both in cultures of mechanically dispersed cell 'miniclusters'[10] and in the intact blastodisc[5–7]. It has also been reported to suppress differentiation and hormone-elicited synthesis of specific proteins in a variety of experimental systems[11–21]. Conversely, 5BdU has been reported to induce differentiation of cultured neuroblastoma cells[22, 23].

However, we believe this to be the first recorded case of both inhibition and stimulation of differentiation in a single experimental system at different concentrations of 5BdU. It remains to be determined whether both effects upon erythropoiesis are due to effects of the nucleoside upon the same or different types of cell present in the heterogeneous cell populations.

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Is Local Antigen Persistence Responsible for the Chronicity of the Experimental Immune Arthritis of the Rabbit?

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Summary. Local antigen trapping in the cartilaginous intra-articular structures can be present without histological evidence of synovitis and is therefore, not necessarily causally related to chronic inflammation.

A chronic arthritis can be induced in immunized rabbits by the intra-articular injection of protein antigens[3, 4]. The systemic humoral response to the antigen seems to be insufficient for the establishment of the chronic inflammatory state unless systemic cell mediated immunity to the antigen is obtained[4, 5]. Two hypotheses have been proposed to explain the chronicity. COOKE et al.[6] have suggested that the antigen persists, locally trapped in the cartilaginous intra-articular structures as an immune complex. This would provide the chronic phlogistic stimulus. GLYNN[7] on the other hand, suggests a locally induced auto-immune process, self perpetuation being the result of an immune response to tissue breakdown products generated during the original antigen induced inflammation. In this communication, we have studied the relationships of host immune status to chronic synovitis and to what has been taken as a measure of antigen persistence[6, 8]. The results suggest that local retention is always a feature of chronic synovitis but that it can be present without histological evidence of chronic inflammation.

Materials and methods. Male New Zealand White rabbits weighing 2 to 3 kg, were used. 2 groups of 5 animals each were immunized by footpad injections of 5 mg of bovine serum albumin (BSA, Sigma) in complete (CFA) or incomplete (IFA) Freund's adjuvant (Difco). 4 weeks

Immune status and chronic synovitis

Immunization (number of animals)	Skin test Score	Antibody ($\log_2$ titer)	Synovitis Score[a]	Retention Ratio[a]
BSA-CFA (7)	2.6±0.4	14.71±1.12	6.6±1.7	88 ±11.6
BSA-IFA (10)	0.6±0.1	11.55±0.47	0.8±0.2	32.7± 7.0
p-value	< 0.001	< 0.01	< 0.001	< 0.001

Values represent mean ± SE *p*-values are for the Student *t*-test.
[a] Ratio of left (BSA injected) to right (saline injected) knees.

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