Differences in in vitro incorporation of tritiated deoxycytidine and tritiated thymidine into human lymphocytes

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Summary. Human lymphocytes stimulated in vitro by PHA or PWM incorporate ^{3}H-CdR into DNA, but less well than ^{3}H-TdR. More ^{3}H-CdR is incorporated in populations enriched for T cells. No ^{3}H-CdR is used by cells stimulated by lipopolysaccharides under circumstances in which ^{3}H-TdR is incorporated. We conclude that human T cells and B cells differ in their ability to use ^{3}H-CdR.

The kinetics of antigenically stimulated rat lymph node cells labeled by tritiated thymidine (^{3}H-TdR) are different from those labeled by tritiated deoxycytidine (^{3}H-CdR) probably due to a deficiency of incorporation of ^{3}H-CdR into late stage B cells[3]. This stimulated us to study human lymphocytes in vitro to see whether a similar difference would provide a new way to differentiate human T and B cells.

Methods. Lymphocytes were collected by sedimentation from heparinized blood obtained at different times from 6 healthy, adult laboratory workers. 10^6 mononuclear cells were cultured in 2.5 ml of Eagle's minimum essential medium (Gibco, Grand Island, N.Y.) containing 100 U/ml penicillin, 100 µg/ml streptomycin, and 20% autologous plasma in polypropylene tubes (Falcon, Oxnard, CA.) at 37 °C in 5% CO_2 and air. The mitogens and concentrations studied were: phytohemagglutinin-M (PHA) 400 µg/ml (Difco, Detroit, Mich.); pokeweed mitogen (PWM), 40 µg/ml, (Gibco); and lipopolysaccharides (LPS) B *E. coli* 026:B6 (B-B6), W *E. coli* 026:B6 (W-B6), and B *E. coli* 0111:B4, (B-B4), 20 µg/ml, and 200 µg/ml (all from Difco). Cultures with PHA were continued for 3 days and all others for 7 days. One or the other nucleoside was then added: 2.5 µCi ^{3}H-CdR or 3.0 µCi ^{3}H-TdR per tube. The cultures were continued 6 h for PHA cultures and 24 h for all other cultures. After the cells were harvested and lysed, the DNA was extracted by standard methods and counted in a liquid scintillation spectrometer, Packard Tri-Carb model 2202. Results are expressed as cpm or as 'stimulation index' (S.I.) i.e.;

$$\frac{\text{cpm test culture}}{\text{cpm unstimulated culture.}}$$

Table 1. Means and SD of the stimulation indices of cultures of human peripheral blood lymphocytes exposed to different stimulants and measured with 2 nucleosides

Stimulant	Dose/ml culture	Numbers of observations	^{3}H-TdR	^{3}H-CdR
PHA	400 µg	11	67 ±40	2.9 ±2.0
PWM	40 µg	9	49 ±21	5.9 ±5.1
LPS:				
W-B6	200 µg	6	4.9± 3.2	1.1 ±0.39
	20 µg	6	3.9± 2.8	1.0 ±0.32
B-B4	200 µg	5	12.8±18	1.5 ±1.2
	20 µg	3	7.4±10	0.87±0.55
B-B6	200 µg	2	4.5± 3.3	1.1 ±0.39
	20 µg	5	3.2± 3.6	1.1 ±0.45

Table 2. Stimulation indices of simultaneous cultures of one subject's peripheral blood lymphocytes which had been separated on Ficoll-Hypaque medium after formation of E-rosettes

	Rosetting layer		Non-rosetting layer	
	^{3}H-TdR	^{3}H-CdR	^{3}H-TdR	^{3}H-CdR
PHA	17	8.4	7.3	1.8
PWM	14	8.6	11.5	2.6

'E' rosettes were prepared by the method of Wybran et al.[4]. 1 ml of a 1% suspension of sheep red blood cells (SRBC) was mixed with 1 ml of a suspension of 10×10^6 washed cells which had been separated from heparinized blood by sedimentation over Ficoll-Hypaque. The suspending medium was Hank's balanced salt solution (Gibco) with 10% fetal calf serum (Gibco). The cells were incubated at 4 °C overnight, gently resuspended, diluted with 2 ml phosphate buffered saline (PBS) at pH 7.2, layered on cold Ficoll-Hypaque, and centrifuged at $500\times g$ at room temperature for 30 min. 'E' rosette-forming cells on the bottom and the cells at the interface were harvested individually, washed twice in PBS at 4 °C, twice in culture medium at 4 °C, and then counted and cultured with PHA or PWM as described. The upper layer of cells always had 15–20% cells which would form E-rosettes when retested.

Results. Addition of ^{3}H-TdR to unstimulated lymphocytes resulted in incorporation of 100–500 cpm. Addition of ^{3}H-TdR after stimulation with PHA produced from 8000 to 52,000 cpm and from 3000 to 46,000 cpm after stimulation with PWM.

Lymphocytes from all 6 normal adult donors responded modestly to stimulation with LPS (table 1). Incorporation of ^{3}H-TdR varied from 700 to 20,000 cpm resulting in S.I. of from 2 to 45. Responses varied with the LPS used, and with different donors.

The incorporation of the 2 nucleosides after stimulation with all mitogens are compared in table 1. Human cells never incorporated as much ^{3}H-CdR as ^{3}H-TdR, but ^{3}H-CdR was utilized by cells stimulated by PHA and PWM. In contrast, the mean S.I. measured by ^{3}H-CdR after LPS stimulation was consistently close to a value of 1, indicating no increase in incorporation.

To determine whether the deficiency of incorporation of ^{3}H-CdR into human blood lymphocytes was due to an excess of unlabeled CdR in the 20% autologous plasma in the culture medium, cells were cultured with PHA and with 5% autologous plasma, 1% human serum albumin, or with 20% autologous plasma which had been dialysed for 16 h against PBS. The use of these alternate protein sources did not increase incorporation of ^{3}H-CdR.

In 4 experiments human peripheral blood lymphocytes forming E-rosettes were cultured simultaneously with cells depleted of E-rosette-forming cells. In all instances, cultures enriched for E-rosette-forming cells incorporated more ^{3}H-CdR than other cultures, but large differences in absolute levels of incorporation prevented statistical analysis. Results of 1 experiment are shown in table 2.

Discussion. These results are consistent with a decreased utilization of ^{3}H-CdR by late stage human B cells in vitro. Although ^{3}H-CdR was utilized less well by human lymphocytes than ^{3}H-TdR, PWM and PHA did stimulate ^{3}H-CdR incorporation into DNA. Cultures enriched for E-rosette-forming cells, presumably T cells[4], had an increased ability to incorporate ^{3}H-CdR after stimulation with PHA. PHA is a T cell stimulator and PWM stimulates T cell dependent B cells, presumably in early stages of development. LPS induced incorporation of ^{3}H-TdR but not of ^{3}H-CdR. Since human lymphocytes are only stimulated by LPS after prior

sensitization[5] and since we were studying laboratory workers with frequent exposure to *Salmonella*, we were probably obtaining an in vitro secondary response by 'memory' B cells.

The differences in incorporation of ^{3}H-CdR and ^{3}H-TdR into DNA in human cells may be due to differences in the intracellular transport systems of the 2 nucleosides as has been shown in pigs[6], but there are also potential differences in enzyme and metabolic pathways. The nucleosides are substrates for different kinases[7] and deaminases[6,8]. Thus our results may be due to potentially identifiable enzyme differences in T and B cells.

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Alpha-fetoprotein in tumor-bearing mice assayed by particle agglutination inhibition

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Summary. Serum alpha-fetoprotein (AFP) in hepatoma BW7756-bearing mice was measured by a new particle agglutination inhibition test employing AFP adsorbed to charcoal particles. The AFP levels and tumor weights showed nearly parallel increases to means of 2633 µg/ml and 5.2 g, respectively, 28 days after implantation.

In investigations of human neoplasms and of experimental animal tumor models, an increasingly important assay is the measurement of AFP in biological fluids (serum, urine, ascites fluid). The highly sensitive immunoenzymatic and radioimmunoassays have proven most effective for humans, whose normal adult blood AFP concentrations range from 1 to 30 ng/ml[1,2]. However, a slightly less sensitive procedure is appropriate for certain strains of mice, whose normal adult concentrations range from 100 to 400 ng/ml[3]. Accordingly, we have developed a rapid, simple charcoal particle card test to detect and monitor serum AFP in tumor-recipient and pregnant mice.

Materials and methods. The sera tested were from 26 Nya:NYLAR and 30 C57L/J normal male and female adult mice, from 13 Nya:NYLAR 15–18-day pregnant mice, and from 57 C57L/J mice with hepatoma BW7756 implants. Sera were collected from tumor-bearing mice sacrificed on days 3, 6, 10, 21 and 28 after implantation. The sera were examined by radial immunodiffusion in agar and by particle agglutination-inhibition tests. The AFP was isolated from the amniotic fluid by DE52 anion exchange chromatography, G-100 Sephadex gel filtration and concanavalin A affinity chromatography[4,5]. Monospecific anti-AFP serum in rabbits was produced as previously described[6]. The purified AFP and the monospecific antiserum were used to prepare a radial immunodiffusion (RID) reference curve[7]. The charcoal particle-AFP (C-AFP) suspension was prepared by admixture of 1 vol of aqueous charcoal (2.5 mg/ml; Hynson, Westcott and Dunning, Baltimore, Md.) with 5 vol of purified AFP (2 µg/ml) in 0.85% NaCl solution and 4 vol of 0.1 M glycine solution (pH 8.2) containing 5 g NaCl and 1 g bovine serum albumin (GBS/BSA). The C-AFP agglutination, using anti-AFP rabbit antiserum, was characterized by dense particle aggregates against a clear background (figure 1). In contrast, C-AFP with control mixtures (normal rabbit serum and GBS/BSA diluent) were a uniform gray.

For the inhibition test, antiserum at 1/100 dilution was mixed with an equal amount of purified AFP or of a heated (56 °C for 30 min) serum specimen. The mixture was incubated at 45 °C for 60 min and then added to C-AFP on a pasteboard card, which was mechanically rotated for 8 min. The minimum detectable concentration of AFP was established by inhibition tests of dilutions of the purified AFP.

Results and discussion. Agglutination-inhibition titrations of purified AFP with antiserum dilutions showed that 0.2 µg AFP completely inhibited agglutination at the 1/100 anti-

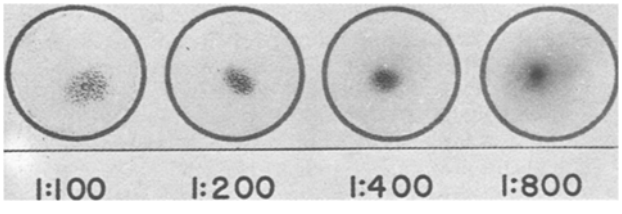

Fig. 1. Titration of rabbit anti-mouse AFP with the C-AFP suspension. 2-fold (1:100–1:800) serial dilutions of antiserum were mixed with the C-AFP suspension. The results were read as 3+, 2+, 1+, and no agglutination.

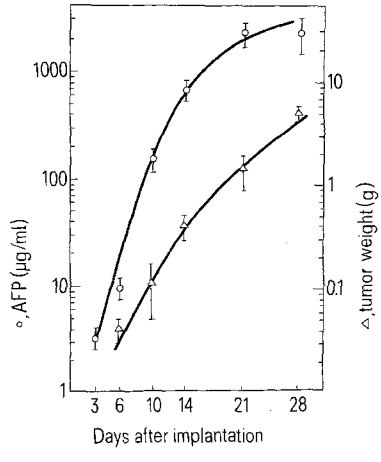

Fig. 2. AFP serum concentrations by charcoal particle test of C57L/J mice after implantation of hepatoma BW7756. Data are shown as mean ± SEM. From 5 to 16 animals were used for each determination.