Effect of prednisolone in vitro on the migration ability of peritoneal macrophages in rats[1]

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Summary. Prednisolone in vitro decreases the random migration of peritoneal macrophages in non-sensitized rats. In sensitized animals cells are sensitive or resistant to prednisolone's inhibitory action, depending on the presence of antigen in the culture.

Glucocorticoid (GC) hormones are therapeutic and experimental agents commonly used in treatment of inflammatory or immunologically mediated diseases, although their effects are complex, diverse and as yet insufficiently elucidated[2]. It is assumed that the anti-inflammatory and immunosuppressive action of GC is partly due to modulation of the circulatory kinetics of various leukocyte populations. The anti-inflammatory effect of GC in vivo was associated with a decrease of cell migration into the inflammatory site[3]. The retention, but not lysis, of recirculating cells in the normal traffic areas, has been suggested as the predominant factor in GC-induced monocytopenia[4] and lymphocytopenia[5]. These alterations in the recirculation pathway of leukocytes may be the consequence of GC action primarily on the cells themselves or primarily on other structures such as vascular endothelium. In vitro studies show that rabbit neutrophil chemotaxis[6], human monocyte chamotaxis[7] and rat T and B lymphocyte random and directed migration[8] can be affected by GC. In this paper we describe the inhibitory effect of prednisolone in vitro on the random migration of rat peritoneal macrophages.

Materials and methods. Experiments were performed on non-sensitized and sensitized Lewis male rats weighing 130±10 g. Sensitization was done either with 100 µg of ovalbumin (Carlo Erba, Milano) in complete Freund adjuvant (Torlak, Beograd) or with 0.5 ml of the same adjuvant. The mixtures were given in all footpads, and animals of the OA-sensitized group were sacrificed 9 days later and those of the BCG-sensitized group 18 days later. The random migration of peritoneal exudate cells (PEC), containing about 80% macrophages as assessed by neutral red staining, was tested using the capillary tube method[9]. PEC, harvested 3–4 days after i.p. injection of sterile paraffin oil, were resuspended in medium (Parker 199, Torlak) with 10% heat-inactivated foetal calf serum (Eurobio, Paris). Erythrocytes were eliminted from PEC suspensions by lysis with 0.83% NH_4Cl solution. The chambers, containing 2 capillaries with cell pellet, were immediately filled with medium alone or with medium containing antigen. As antigens were used OA (100 µg/ml) in OA-sensitized group, and 50 TU of PPD (Statens Seruminstitut, Copenhagen) in BCG-sensitized group. Prednisolone (6-methylprednisolone-hemisuccinate, Hoechst) was used in vitro in the concentration noted in the results. The average migration areas of 4 replicates, determined after 20 h of incubation of 37 °C, were used for the calculation of the migration index (MI = test area/control area × 100). Cell viability assessed by trypan blue exclusion at the end of the culture period. The percentage of cytolysis was calculated by the equation (a-b)/b × 100, (a = concentration of viable cells in control cultures, b = concentration of viable cells in test cultures).

Results. The incubation of non-sensitized cells with 10^{-6} M prednisolone insignificantly reduced their migration areas, whereas with the pharmacological concentration of the drug (10^{-5} M) this reduction was more cleary expressed and statistically significant ($p<0.05$). In a condition of unsecessful sensitization (OA-group) prednisolone exhibited a dose-dependent and highly significant inhibition of migration both in the absence and in the presence of antigen in the culture (from $p<0.005$ to $p<0.001$). In contrast, in the PPD-group prednisolone demonstrated a strong inhibitory effect on cell migration only in the absence of antigen ($p<0.005$ and $p<0.001$). In the presence of antigen the cells were very resistant to the inhibitory effect of the drug, as moderate suppression was obtained only at a supraphar-macological concentration of prednisolone ($p<0.05$). The consequence of this effect was abolition of migration inhibition. The experiments were performed twice in the PPD-group with similar results. Cytolysis (2.32–2.81%) was not responsible for the observed reduction of macrophage migration.

Discussion. Cell migration represents a complicated and only partially-defined process of surface and intracellular events. This vitally important function was affected by GC in vitro. The concentration of 10^{-5} M hydrocortisone in vitro inhibited human peripheral blood monocyte chemotaxis[7]. A similar effect was demonstrated for rabbit neutrophil chemotaxis[6]. Beer and Center reported that hydrocortisone, independent of cell lysis, inhibited the random and directed migration of rat splenic T and B lymphocytes, and human peripheral blood T lymphocytes in vitro[8]. Our results extend these findings to the random migration of peritoneal macrophages in normal and sensitized rats. The

Influence of prednisolone in vitro on migration of peritoneal macrophages in non-sensitized and sensitized rats

Sensitization	Prednisolone	Migration areas (±SE) with antigen Absent	Present	Migration indexes (±SE)
-	-	22.33±2.34		
	1×10^{-6} M	18.85±1.83		
	-	17.34±1.56		
	1×10^{-5} M	11.76±1.33*		
OA	-	18.68±1.37	16.46±1.28	86.12±5.52
	1×10^{-5} M	10.82±0.79**	9.78±0.73**	85.21±4.33
	1×10^{-4} M	7.05±0.34***	7.34±0.53***	101.74±6.03
PPD	-	23.45±1.83	13.43±0.96	55.82±4.75
	1×10^{-6} M	21.17±2.01	13.62±1.10	61.29±4.19
	1×10^{-5} M	15.33±1.24**	12.26±0.75	86.73±6.52
	1×10^{-4} M	11.75±1.05***	10.42±0.95*	90.31±6.33

The number of animals per group was 5–6. * $p<0.05$; ** $p<0.005$; *** $p<0.001$ by 2-tailed Student's t-test.

suppression of migration was obtained at concentrations similar to those used in the papers cited. We reported a similar result previously in guinea-pigs[10].

GC in vitro also affect many biochemical functions of macrophages. However, the protein synthesis[11] and enzyme secretion[12] were inhibited with physiological or smaller concentrations of drug, indicating a different sensitivity of various cell functions to GC action. The appearance of resistance to prednisolone inhibitory action in the PPD-group is in accordance with ideas postulated by Claman[13] and Baxter and Harris[14]. They consider that lymphoid cells may be either steroid sensitive and resistant, according to their stage of activation in the course of the immune response. All the above-mentioned data about GC effects on migration of various cell types in cortisone-sensitive (rabbit, rat) and cortisone-resistant species (man, guinea-pig) may indicate interference with the basic mechanism involved in cell migration. At the same time, the GC-induced defects in cell movement may contribute to reduced cell defense during GC therapy.

1 This work was supported by the grant of Republic Reseach Fund of Serbia.
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0014-4754/83/101133-02$1.50 + 0.20/0

Incorporation of 5-bromodeoxyuridine in the total and ribosomal DNA of synchronously dividing chick embryo fibroblasts[1]

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Summary. The pattern of 5-bromodeoxyuridine incorporation into ribosomal DNA is quantitatively different from that for total DNA. It is concluded that 5-bromodeoxyuridine incorporation along the DNA chain is not a random process.

5-Bromodeoxyuridine (BrdUrd) is a nucleotide analogue of thymidine (dThd) which is easily incorporated into DNA in place of dThd. This analogue has been widely used in studies on DNA replication: its incorporation into DNA produces an increase of the buoyant density in cesium chloride gradients which allows the easy separation of replicated DNA from non-replicated DNA. There is an abundant literature dealing with the effects of BrdUrd on cellular metabolism during the differentiation process (for review see Rutter et al.[4]). Moreover, it was shown that some families of moderately repeated DNA from different organisms were preferentially substituted by BrdUrd when low concentrations of the analogue were used[5-8]: the substitution of dThd by BrdUrd was not a random process. For these reasons, we set out to make a comparative study of the extent of BrdUrd incorporation, depending on the concentration of the analogue in culture medium, into chick total DNA and ribosomal DNA (rDNA), the latter representing a well-defined, moderately repeated fraction of DNA. Nevertheless, as chick rDNA content is only 0.02–0.12% of bulk cellular DNA[9,10], i.e. about 0.25 to 1.5% of repeated and intermediate DNA sequences[11], our results could not be extended to the overall repeated and intermediate DNA. However, the extent of rDNA substitution, compared to bulk DNA, might represent an experimental approach to demonstrate a non-random incorporation process of the analogue along the DNA chain.

This paper describes the experimental strategy used to measure the extent of BrdUrd incorporation, depending on its concentration in the culture medium, and shows that the pattern of rDNA substitution differs notably from that of bulk DNA.

Materials and methods. Cell cultures. Primary cultures of chick embryo fibroblasts were prepared as described by Temin and Rubin[12]. The synchronization procedure was as described by Goldé et al.[13]. For the determination of total DNA substitution, labeled BrdUrd (5 µCi/plate) was added, at the time of release of mitosis, at concentrations ranging from 0 to 10 µg/ml. For the determination of rDNA substitution, unlabeled BrdUrd was added in the same concentration range. For the preparation of (^{3}H)rRNA, cultures were supplemented with ($^3H_5$)-uridine (500 µCi/plate) during 1 cell cycle and chased for 4 h with unlabeled uridine 100 times more concentrated than the labeled nucleotide.

DNA preparation. After 1 cell cycle, cells were killed by addition of 1.4×10^{-3} M NaF, and scraped into phosphate-buffered saline buffer. DNA was extracted according to Hughes et al.[14] and subjected to neutral CsCl density gradient centrifugation (refractive index adjusted to 1.4010, 28,000 rev/min for 95–100 h at 20 °C, rotor Beckman 60 Ti). After centrifugation, gradients were harvested into 22–24 fractions. For the determination of total DNA substitution, the radioactivity and the absorbance at 260 nm (A_{260}) of each fraction were assayed. Fractions corresponding to substituted DNA (selected on the basis of the radioactive profile) were pooled. For the determination of rDNA substitution, each fraction was assayed for A_{260} and was further processed as described under DNA-^{3}H-rRNA hybridization'.

^{3}H-rRNA preparation. Labeled chick rRNA was prepared as previously described[15].

DNA-^{3}H-rRNA hybridization. An aliquot (100 µl) from the fractions of each gradient was denatured and fixed on a