INTERACTION BETWEEN CHALONES AND GLUCOCORTICOID HORMONES DURING REGULATION OF CELL DIVISION

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A fundamental problem in the regulation of cell division in tissues is the character and direction of interaction of factors at the tissue and organism levels. In the first studies of the action of chalones on the cell cycle it was suggested that stress hormones (adrenalin and glucocorticoids) are cofactors of chalones. It was subsequently shown that the inhibitory effect of chalones on cell proliferation can be exhibited even without adrenalin [12]. In an investigation on epithelia of epidermal type no direct relationship could be found between the action of adrenalin, hydrocortisone, and epidermal chalone on mitotic activity [11].

It was decided to study whether any connection exists between the action of glucocorticoid hormones and chalones on tissues which are targets for adrenocortical hormones. One target organ for them is the liver [8]. It was shown previously that hepatoma 22a, a malignant tumor of the liver, preserves sensitivity to hydrocortisone [1] and to a chalone isolated from the liver of intact rats [2].

EXPERIMENTAL METHOD

Male C3HA mice weighing 23-25 g were used. All the mice were inoculated with hepatoma 22a (the solid variant) by subcutaneous injection of $5 \cdot 10^5$ tumor cells in 0.3 ml physiological saline. The experiment was carried out on the 9th day after inoculation. The animals were divided into nine groups. Mice of group 1 served as the control. Mice of groups 2 and 3 received a single intraperitoneal injection of hydrocortisone acetate (from Gedeon Richter, Hungary), at 3 p.m. on the day of the experiment, in a dose of 15 mg/100 g body weight. Mice of groups 4 and 5 received daily injections of hydrocortisone for 7 days. Mice of groups 6 and 7 underwent bilateral adrenalectomy 5 days before the experiment. The mice of these groups were given 0.9% NaCl solution to drink. Mice of groups 3, 5, 7, and 8 were given chalone-containing liver extract from intact rats in a dose of 15 mg per mouse at 3 p.m. The chalone was isolated by the method of alcoholic precipitation in [9] with certain modifications [2]. The preparation thus obtained contained 50% of proteins.* An alcoholic precipitate obtained within the range of concentrations of ethyl alcohol of 55-81% is known to contain G_1- and G_2-chalones [4].

To assess objectively the mitotic activity all animals were given an intraperitoneal injection of colchamine (colcemid) in a dose of 5 mg/kg body weight. Colchamine was used because of the need to prevent any effect of possible changes in the duration of mitosis on the results, for epidermal chalone has been shown to lengthen the duration of mitosis [10]. To record DNA-synthesizing cells in the hepatoma, ^{3}H-thymidine was injected into all the animals 1 h before sacrifice, in a dose of 1 μCi/g body weight (specific activity 23 Ci/mmole). The mice were killed at 9 p.m., i.e., 6 h after injection of chalone-containing extract. Pieces of tumor for histological investigation were fixed in Carnoy's fluid and auto-

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TABLE 1. Changes in Cell Proliferation in Hepatoma 22a Following Injection of Hepatic Chalone Together with Hydrocortisone and Adrenalectomy

Group of animals	Experimental conditions	No. of mice	MI_{col}, %	% of inhibition	RI, %	% of inhibition
1	control	10	58,7±4,0	—	209,9±4,9	—
2	Hydrocortisone, singly	10	38,7±3,0	34,1	149,6±8,4	28,7
3	Hydrocortisone, singly + chalone	10	22,9±1,9	61,0	34,7±3,2	83,5
4	Hydrocortisone, repeatedly	13	29,3±2,4	50,1	153,3±8,0	27,0
5	Hydrocortisone, repeatedly + chalone	12	16,2±0,9	72,4	34,2±2,7	83,7
6	Adrenalectomy	8	58,4±2,2	—	196,4±5,9	—
7	Adrenalectomy + chalone	8	32,2±1,9	45,2	116,8±13,1	40,5
8	Chalone	10	21,3±1,2	63,7	30,9±3,5	85,3

radiographs were prepared. Mitotic activity (the number of mitoses blocked by colchamine in 3 h — MI_{col}) and the index of labeled nuclei (radioactive index — RI) were determined by examining 6000-8000 cells in the preparations in each case and calculating these indices in promille.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1. MI_{col} was reduced by 2.7 times and RI by 6.8 times below the control values 6 h after injection of the chalone. Injection of hydrocortisone caused a much weaker, although still significant effect. The degree of reduction in the number of DNA-synthesizing nuclei in the hepatoma, incidentally, was the same irrespective of whether the chalone was injected once or more than once. Meanwhile the number of c-mitoses was reduced more after repeated injection of the hormone than after a single injection ($P = 0.02$). A combination of hydrocortisone with chalone had the same inhibitory action on DNA synthesis as injection of the chalone alone. Cells in the premitotic phase of the cycle were more sensitive to the combined action of repeated injections of hydrocortisone and chalone than to a single injection of the two preparations.

It follows from the results that an increase in the chalone concentration in the body against the background of the natural glucocorticoid hormone level causes sharp inhibition of proliferation of tumor cells. However, for the inhibitory action of the chalones to be completely manifested, a definite minimal level of adrenocortical hormones in the blood is evidently necessary. This follows from the data on the level of proliferation in the hepatoma in adrenalectomized animals. Adrenalectomy in rodents does not lead to complete disappearance of glucocorticoids because of the presence of accessory adrenals in these animals. At the times after adrenalectomy chosen in the present experiments the blood 11-HCS level was about 34% of normal [4]. Under these conditions the intensity of proliferation was unchanged both in the mouse liver [5] and in hepatoma 22a (Table 1), because of the high sensitivity of the cells of these tissues to glucocorticoids. However, the inhibitory effect of hepatic chalone when injected into adrenalectomized animals was reduced. After injection of the chalone into adrenalectomized mice the degree of inhibition of mitotic activity was 54.2%, compared with 63.7% in the control ($P < 0.001$). The index of labeled nuclei was lowered by 44.4%, compared with 85.3% in the control ($P < 0.001$). For the action of the chalone to be manifested, a definite optimal ratio between glucocorticoid hormones and chalones is thus necessary. Consequently, in hepatoma 22a (and also, we may suppose, in the liver) synergism of action of glucocorticoids and chalones in the regulation of cell proliferation is clearly manifested. However, the writers showed previously [3, 6] that repeated injections of hydrocortisone lead to an increase in mitotic activity in epithelia of epidermal type. This fact suggests that the character and degree of interaction of glucocorticoid hormones and chalones may vary depending on the type of tissue.

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KINETICS OF PRODUCTION OF MACROPHAGE MIGRATION INHIBITION FACTOR IN MIXED CULTURES OF LYMPHOCYTES AND TUMOR CELLS

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The continuous renewal of most tissues of the multicellular organism, taking place through intensive cell division, is responsible for the high frequency of appearance of mutant cells, proliferation of each of which may lead to disturbance of vitally important functions of different organs or to the development of a malignant tumor [6]. The diagnosis and timely elimination of foreign cells and supervision of genetic homeostasis of the organism are functions of the immune system. The concept of immunologic surveillance was formulated by Burnet in the mid 1960s [6], but the nature of the effector cells of immunologic surveillance has not yet been precisely established. The most likely candidates for the role of these cells, capable of reacting to a foreign antigen without previous immunization, are the natural killers (NK cells) [8] and natural producers of macrophage migration inhibition factor (MIF) [12], lymphocytes potentially capable of activating the antitumor resistance of macrophages [13]. The writers showed previously that T cells which are producers of MIF can distinguish between mutant H-2 antigens and original H-2 antigens during development of an immune response *in vivo* [3] and *in vitro* [5].

The object of the present investigation was to study the ability of nonimmune MIF producers to react to antigens of allogeneic and syngeneic tumors during the first days of mixed culture of lymphocytes and tumor cells (MCLT) *in vitro*. The preliminary results on recognition of syngeneic tumor antigens were published previously [2].

EXPERIMENTAL METHOD

Spleen cells of normal mice of lines C57BL/6, abbreviated to B6 (Haplotype $H\text{-}2^b$); C57BL/10Sn, abbreviated to B10 ($H\text{-}2^b$), DBA/2 ($H\text{-}2^d$), and CBA ($H\text{-}2^k$), were used as reacting cells in MCLT. The stimulating cells were tumor cells irradiated in a dose of 5000-10,000 rads, obtained from sarcoma MCh-11, T-lymphoma EL-4, and mastocytoma P-815, maintained in the writers' laboratory by regular passages in mice of lines B10, B6 and DBA/2 respectively. Reacting cells numbering 5×10^6 were mixed with 1×10^5 stimulating cells and incubated in a final volume of 2 ml of medium RPMI-1640 (from Flow Labs, England) with the addition of 10% embryonic calf serum (from Gibco, England), 2 mM L-glutamine (from Flow Labs), 0.005 M HEPES buffer, 5×10^{-5} M 2-mercaptoethanol, and antibiotics. Lymphocytes of syngeneic spleens,

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