Attempt to Alter Antigenic Structure of Normal Tissues by Treatment with Dimethyl-Triazeno-Imidazole-Carboxamide in vivo[1]

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Summary. The antigenic structure of normal skin and spleen cells has been investigated following in vivo treatment with the compound DIC. In experiments involving skin grafting in the normal and sensitized host, cross sensitization with a DIC-antigenic lymphoma and ^{3}H-thymidine incorporation by lymphocytes cultured with DIC-treated spleen cells, new antigens on DIC-treated tissues were not demonstrated.

It was previously demonstrated that new antigenic specificities may be induced in lymphoma cells by appropriate treatment in vivo with antineoplastic drugs[4–12]. For this purpose, the anticancer compound 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DIC) is among the most active agents. The widespread treatment of autoimmune diseases with immunosuppressive antineoplastic compounds has strengthened the interest in determining whether the antigenic structure of normal tissues could be altered after a course of administration of DIC.

Materials and methods. Inbred DBA/2 Cr, C57Bl/6J, C3H/f or hybrid (Balb/c ♀ × DBA/2 ♂) CDF_1 female mice were used. Skin grafting was carried out according to the classical technique of Billingham[13]. Donor skin was excised from the animal, connective and fat tissue removed, and placed on a small area (5 mm diameter) in the mammary region of the recipient host. The graft was fixed in position with adhesive strips, covered with cotton and gauze and maintained in situ by plastic bandage.

Table I. Skin of DIC-treated CDF_1 mice grafted to normal or immune CDF_1 animals

Donor	Recipient immune to	T/G[a]
CDF_1	—	24/26
CDF_1-DIC[b]	—	13/16
CDF_1-DIC[c]	—	13/15
CDF-DIC[b]	CDF_1-DIC	7/8
CDF-DIC[c]	CDF_1-DIC	8/9
CDF_1-DIC[c]	L1210/DIC	14/16
CDF_1	L1210/DIC	20/21
C3H	—	0/8

[a] T/G, graft takes/total number of grafts. [b] DIC-treated CDF_1 mice treated for 30 days. [c] DIC-treated CDF_1 mice treated for 60 days.

Table II. Leukemic cell challenge i.p. in immune, X-irradiated CDF_1 mice

Immune to	Tumor challenges		MST[a]	D/T[b]
CDF_1-DIC	L1210/DIC	10^4	14	8/8
CDF_1-DIC	L1210/DIC	10^6	9	8/8
L1210/DIC	L1210/DIC	10^4	—	0/8
L1210/DIC	L1210/DIC	10^6	—	0/8
CDF_1-DIC	L1210	10^4	10	8/8
L1210/DIC	L1210	10^4	10	8/8

[a] MST, median survival time (days). [b] D/T, dead mice/total mice.

On day 8 the skin graft was uncovered and the borders were checked daily for 30 days. For the following 6 months a monthly check was made.

L1210/DIC, a subline of L1210 leukemia originated following DIC treatment in vivo, was maintained by serial passage i.p. in immunosuppressed (Cyclophosphamide 200 mg/kg i.p. 24 h before leukemic challenge) CDF_1 mice[14]. Challenge with 10×10^6 viable L1210/DIC cells i.p. or inoculation s.c. of 10 skin pieces was used to sensitized animals. Mixed lymphocyte culture (MLC) was carried out according to the method described by Bach[15]. Mitomycin C (30 µg/ml) was used to block ^{3}H-thymidine incorporation by stimulator cells.

Results. Skin grafts of DIC-treated mice (100 mg/kg i.p. daily, 5 treatments a week for 30 or 60 days) were accepted by the syngeneic CDF_1 host. In contrast, C3H skin allograft were rejected (Table I).

The CDF_1 animals previously sensitized to either skin of DIC-treated animals, or to the L1210/DIC antigenic subline, failed to demonstrate second set rejection of CDF_1-DIC skin transplants (Table I). Thus, new transplantation antigens were not demonstrated on DIC-treated skin tissues.

The primary but not the secondary immune response is inhibited by X-rays[16]. Animals sensitized to DIC-treated skin or to L1210/DIC were X-irradiated (400R)

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Table III. Lack of stimulation of CDF_1 spleen cells by DIC-treated spleen cells in MLC

Stimulated	Stimulator	Expected cpm ± SE	Obtained cpm ± SE	Stimulation index
CDF_1	CDF_1	—	6892 ± 820	1
CDF_1	CDF_1-DIC	4190 ± 382	3962 ± 460	0.9
CDF_1	CDF_1-DIC_m	3061 ± 247	3346 ± 104	1.1
CDF_1	$C57B1/6_m$	3843 ± 106	14882 ± 1037	3.8
CDF_1	PHA	3446 ± 128	56424 ± 1891	16.3

24 h before challenge with L1210/DIC viable cells. L1210/DIC leukemia grew progressively and killed animals previously sensitized to DIC-treated skin, but was rejected in mice that had been sensitized to L1210/DIC cells (Table II).

The results of the above experiments indicate that DIC-treated skin was ineffective in sensitizing syngeneic mice or in cross-reacting with DIC-induced antigens on leukemic cells. CDF_1 spleen cells, incubated with spleen cells from DIC-treated CDF_1 mice, have not been stimulated (Table III).

The failure to detect DIC-induced 'stimulating' antigens on spleen cells is in contrast to the stimulation observed by DIC transformed leukemic cells[17]. In studies not reported here, DIC-treated spleen cells did not stimulate allogeneic lymphocytes. DIC treatment might deplete B lymphocytes which have the property of acting as stimulators of T lymphocytes in mixed lymphocyte culture[18]. If this were the case, spleen of DIC treated mice might contain responder T lymphocytes predominantly.

Discussion. The nature of drug-induced antigen(s) on tumor cells has not been investigated extensively. The studies have been concerned primarily with the occurrence of new tumor transplantation antigens. There are two major aspects of DIC activity: a) DIC induced antigen(s) in neoplastic cells. b) In the current experiments normal tissues were apparently not altered by chronic treatment with DIC which is an immunosuppressive drug. No evidence could be obtained that new antigens were expressed on normal skin following DIC treatment in vivo. The lack of response of CDF_1 lymphocytes could be interpreted in two possible ways: a) there was no antigenic alteration detectable by the MLC method for the DIC-treated spleen cells; b) if DIC treatment depleted B lymphocytes, this might still leave open the possibility that T lymphocytes could be altered by DIC treatment, although not detectable in MLC.

Therefore the hypothesis that an immunosuppressive treatment such as with DIC might induce a new autoimmune disease, clinically apparent after the discontinuation of the therapy, does not seem to be supported. The possibility that DIC may deplete preferentially B lymphocytes requires further investigation. This property would be of general interest in therapeutic research.

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Mitochondrial Derivation of Centrioles in Some Endocrine Adenomas

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Summary. Fine structural studies of various endocrine adenomas indicate that mitochondria may serve as progenitors of centrioles and cilia.

Centrioles, known to play a fundamental role in mitotic division, occur with varying frequency in different cell types and exhibit uniform, easily recognizable features by electron microscopy[2]. While studying the fine structure of surgically removed human tumors arising from various endocrine glands, several adenomas were found which contained numerous structures believed to represent forming centrioles in various phases of development providing an opportunity to investigate the subcellular aspects of centriologenesis[3].

The material consisted of 17 chief cell adenomas of the parathyroid, 7 sparsely granulated growth hormone cell adenomas, 4 mixed adenomas composed of growth hormone cells and prolactin cells, 3 undifferentiated cell adenomas of the pituitary and 1 pheochromocytoma arizing from the adrenal medulla. These tumors were selected from 76 cases because they contained numerous centrioles.

Small pieces of tumor tissue were fixed immediately after their surgical removal in 2.5% glutaraldehyde in 0.15 *M* Sorensen's buffer, postfixed in 1% osmium tetroxide in Millonig's buffer, dehydrated in graded ethanol and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and investigated with a Philips 300 electron microscope.

While attempting to reconstruct the various steps of centriole formation from the electron micrographs, a hitherto undetected sequence of events seemed to emerge: centrioles appeared to arise from mitochondria. Firstly, mitochondrial cristae disappeared and fibrillar-granular material accumulated at one pole of the mitochondria. The rest of the internal compartment was usually occupied by a clear vacuole (Figure 1). The double mitochondrial membranes exhibited increased electron density and, by further accumulation of fibrillar-granular substance, mitochondria gradually transformed into procentriolar bodies (Figures 2 and 3). Then the double membranes appeared to disintegrate followed by an asymmetrical division of the electron dense material (Figure 4). As-

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