the antibodies. Thin sections were cut on a LKB Ultrotome, and examined in a Hitachi HU-11C type electron microscope without electron staining.

In thin sections of the EAT cells treated with SLE sera, the nuclei were stained strongly and stood out with sharp demarcation against electron lucent cytoplasm at low magnification (Figure 2). At higher magnification peroxidase activities were mainly localized in the well defined areas corresponding to perinuclear and nucleolus-associated chromatin, whereas interchromatinic zones and nucleoli remained unstained (Figure 3). Clusters of tiny granules with low electron density, corresponding probably to interchromatin granules, were observed in these interchromatinic zone. Perichromatin granules could not be identified since they were located closely to chromatin.

In the cytoplasm of SLE sera-treated cells, the intense antigenicity was occasionally found in the cisternae of the endoplasmic reticulum, though in small numbers (Figure 3).

In the cells treated with control sera, no reaction products were seen in the nuclei (Figure 1). The endogeneous peroxidase activity were seen in the homogeneous, spherical granules, approximately 0.5 μm in diameter, and in lipid droplets[12].

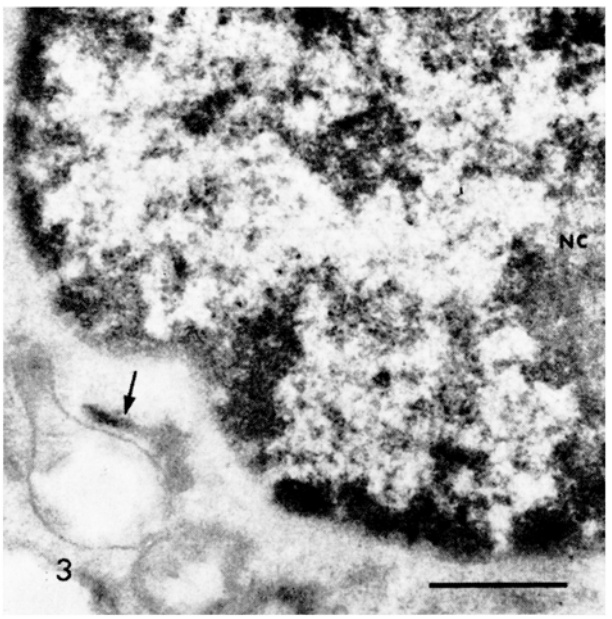

Fig. 3. Part of EAT cell treated with SLE sera, demonstrating stained chromatin and unstained interchromatinic zone. In the interchromatinic zone, fine granules of low density are visible. An arrow indicates staining of the cisternae of endoplasmic reticulum. NC, nucleolus. ×18,000.

The present results clearly show that the antigenicity associated with antinuclear factors are localized in the chromatin, and not in nucleolus or interchromatinic zone, suggesting that nuclear DNA or DNP is involved as antigens in this procedure in the light of the current concepts on the nuclear ultrastructures[13]. This is, also, compatible with the immunological assays of SLE sera by the purified antigenic substances[14–18]. In addition to the currently recommended techniques[13] including enzymatic digestion and chemical extraction, the ultrastructural immunohistochemistry employed here will be suitable for further elucidation of the topology and ultrastructure of nuclear components.

Anticytoplasmic factors in SLE sera have been described as species-nonspecific antibodies[19,20]. The antigenic constituents reacting with these anticytoplasmic antibodies have not yet been characterized. The present results may show that the intracisternal moiety of endoplasmic reticulum is one of the responsible antigens for the antibodies.

Zusammenfassung. Nach indirekter, peroxidasekonjugierter Antikörpermethode wurden elektronenmikroskopisch Ehrlich-Aszitestumorzellen geprüft und am Chromatin solcher Zellen das Antigen gegen den antinukleären Faktor in Seren einiger systemisch Lupus-erythematosus-Erkrankter gezeigt.

M. MACHIDA[21] and M. HOSHINO[22]

The Second Department of Internal Medicine, Nagoya University School of Medicine, and Laboratory of Ultrastructure Research, Aichi Cancer Center Research Institute, Tashiro-cho, Chikusa-ku, Nagoya (Japan), 15 June 1970.

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[21] The Second Department of Internal Medicine, Nagoya University School of Medicine, Showa-ku, Nagoya (Japan).
[22] Reprints reguest: M. HOSHINO, Laboratory of Ultrastructure Research, Aichi Cancer Center Research Institute, Chikusa-ku, Nagoya (Japan).

On the Immunodepressive Action of Adriamycin

Adriamycin is an anthracycline antibiotic isolated in 1967 from mutant of *Streptomyces peucetius*[1,2]. Its antineoplastic action had been studied on ascitic and solid tumours induced experimentally in mice and rats[3–5] and recently in man on different neoplasms and in leukaemias of children and adults[6–10].

Recent studies[11] have demonstrated that adriamycin is able to inhibit 'blastic' transformation induced by phytohaemagglutinin (PHA) in human lymphocyte cultures and interfers with the mechanism of cellular DNA and RNA synthesis[12].

Material and methods. Male Swiss Cobs mice, weighing 20–22 g, were employed. The animals were immunized i.v. with 0.5 ml of a 2% suspension of sRBC in physiological saline. Doses and treatment schedules employed are reported in the Table. 10 animals for each experi-

Treatment schedule

No. of experiment	Dose (mg/kg/die)	No. of animals	Route of administration	Total dose of drug (mg/kg)	Days of administration with respect to immunization (day 0)
1	1.4	40	i.p.	5.6	—3, —2, —1, 0
	1.4	40	i.p.	9.8	—3, —2, —1. 0, +1, +2, +3
	1.4	40	i.p.	5.6	0, +1, +2, +3
	–	40	–	–	
2	1.5	40	i.v.	9.0	—3, —2, —1 +1, +2, +3
	2.5	40	i.v.	15.0	—3, —2, —1 +1, +2, +3
	–	40	–	–	
3	3.0	50	i.v.	9.0	—3, —2, —1
	3.0	50	i.v.	9.0	+1, +2, +3
	1.5	50	i.v.	9.0	—3, —2, —1 +1, +2, +3
	–	50	–	–	

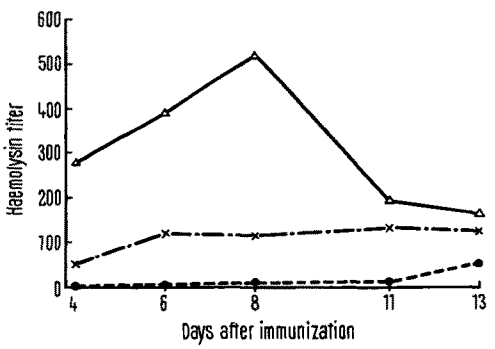

Fig. 1. Effect of adriamycin treatment on the primary response of mice, immunized with sRBC. △—△, control group. ×—×, adriamycin 1.5 mg/kg/die i.v.; ·–·–·, adriamycin 2.5 mg/kg die i.v..

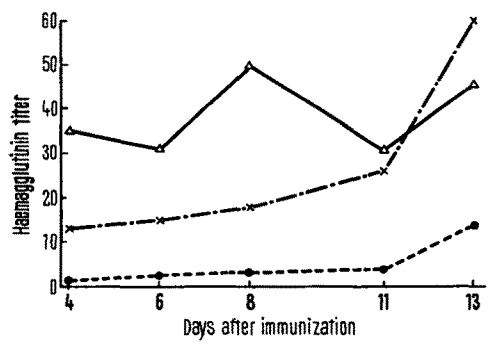

Fig. 2. Effect of adriamycin in treatment on the primary response of mice immunized with sRBC. △—△, control group. ×—×, adriamycin 1.5 mg/kg/die i.v.; ·–·–·, adriamycin 2.5 mg/kg/die i.v..

mental group were sacrificed at different intervals of time after immunization and blood samples (1 ml) were obtained from each animal by means of cardiac puncture.

The hemolytic and hemagglutinating titers carried out using the microtiter equipment, are expressed as the reciprocal of the geometric means of the highest dilution of serum capable of causing hemolysis and hemagglutination of 50% of a 0.5% sRBC suspension. Means were calculated on 10 different replications.

The immunodepressive action of adriamycin was evaluated by calculating the suppression index (SI), which expresses the ratio between the mean antibody titer of treated mice and the control ones according to DIETRICH[13], as well as by means of statistical analysis of variance (Anova).

Results. The experimental results, reported in Figures 1, 2 and 3, demonstrate clearly that adriamycin administered i.v. depressed the synthesis of circulating antibodies, hemagglutinin and hemolysin, in the treated animals, whatever the treatment schedules employed.

The immunodepressive action of this substance appeared to be related to the total dose of the drug administered. Also the i.p. treatment with this drug produces a clear immunodepressive effect on the appearance of humoral antibodies, but it causes local alterations. Among the various treatment schedules employed, the greatest activity was observed when the substance was administered for 3 days after immunization (Figure 3).

Generally the action was most marked 4, 6 and 8 days after administration of the antigen and the most significant immunodepressive effect was observed when the total dose 15 mg/kg had been reached. In this case, an almost total inhibition of the circulating antibodies was seen (Figures 1 and 2).

Statistical analysis showed that the differences of values observed between the control animals and those treated intravenously were significant at the 4th, 6th and 8th day after immunization, at a level of 5% or even lower. In our experiments animal mortality was never observed even with the highest doses, which caused only a slight decrease in body weight, becoming normal after some days following treatment.

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Conclusions. The experiments carried out showed that adriamycin significantly depressed the level of circulating antibodies; its action depends on the total dose of the drug administered. The highest immunodepressive effect was observed when the treatment followed the antigen administration. This finding suggests that adriamycin does not interfere with the very early steps of immunological response.

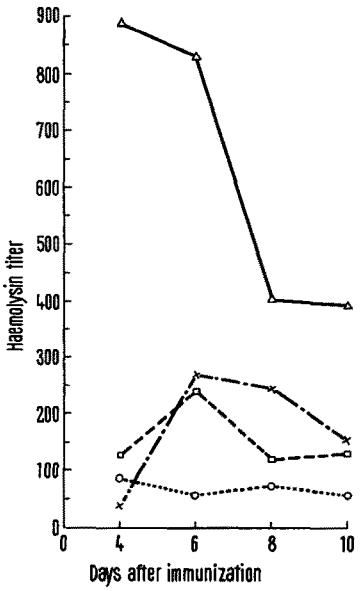

Fig. 3. Effect of adriamycin on the primary response of mice immunized with sRBC. The total dose of adriamycin administered was 9 mg/kg for the 3 different experimental groups of mice. △—△, control group. ×—×, treatment at days —3, —2, —1 (3 mg/kg/die i.v.). □—□, treatment at days —3, —2, —1, +1, +2, +3 (1,5 mg/kg/die i.v.). ○—○, treatment at days +1, +2, +3 (3 mg/kg/die i.v.). O, day of immunization.

The immunodepressive effect of this antibiotic probably depends on its action on lymphatic tissue and on highly proliferating cell systems[14]. In fact, as chronic toxicity studies on the dogs demonstrated[15], adriamycin causes a marked reduction of lymphatic tissue of thymus, lymphnodes, spleen and provokes a blood lymphocytopenia. Besides, adriamycin, interfering with nucleic acid synthesis[12], has an inhibiting effect on bone marrow cells, and on blood lymphocytes stimulated by PHA[11]; a similar action can be supposed on immunocompetent cells after antigenic stimulation.

Riassunto. Studi sperimentali hanno messo in evidenza che l'adriamicina possiede un'azione immunodepressiva in quanto determina una inibizione significativa del titolo degli anticorpi emolitici ed emoagglutinanti in topi immunizzati con globuli rossi di montone.

A. M. ISETTA, C. INTINI and M. SOLDATI

Farmitalia S.A., Istituto Ricerche di Base, Via dei Gracchi 35, I-20146 Milano (Italia), 27 July 1970.

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Observations on Non-Specific Reactions to Tuberculin in Sheep and Goats with *Corynebacterium ovis*

The problem of non-specific reactions to tuberculin amongst cattle has been very commonly met with in many countries where the campaign for tuberculosis eradiation has been taken up on a large scale. In areas relatively free from bovine tuberculosis, the problem of non-specific reactions was mostly confronted. Various causative organisms, namely actinomyces, actinobacillus, Johnes' *Bacillus faciola*, brucella species, and pyogenic infections have been reported to elicit non-specific reactions to tuberculin in cattle (KLEEBERG[1], RUSFORD[2], and TRANTWEIN[3]). CANHAM[4] also reported sensitivity to tuberculin in guinea-pigs infected with the *Corynebacterium pyogenes*. But from the literature it is evident that non-specific sensitization to the tuberculin in ovines and caprines has not been reported so far.

The present paper includes the study of a naturally infected goat and a sheep which reacted to tuberculin in routine testing of the herds. These reactors were sacrificed for the isolation of the causative organisms and their characterisation.

Material and methods. Case No. 1. An indigenous goat No. 426 of Katula goat farm at Indian Veterinary Research Institute, Mukteswar-Kumaon, was subjected to double intradermal test with tuberculin and johnin simultaneously. The animal reacted to the tuberculin but not to johnin. This goat was sacrificed and on thorough postmortem examination an abscess of 2″ diameter was found in the diaphragmatic lobe of right lung which had thick hard capsule and was filled with soft caseated yellowish pus. No other lesions were seen in other visceral organs. Smears were also made from ileo-caecal gland for detection of Johnes' bacillus.

A direct attempt was made to isolate culture from the abscess. The smears were made and stained with Zeihl-Neelson's and Gram's stains. The pus material along with fibrous capsule was triturated aseptically in sterile saline and was inoculated into 2 guinea-pigs by i.m. route, 3 fowls by i.v. route and 2 kids by subcutaneous route in neck. Each animal was inoculated with 1 ml of suspension.

Kids were also infected with new isolates by different routes. Infected animals were tested with tuberculin at varying intervals of time.

Case No. 2. A Bikaneri sheep of the Genetics Division, I.V.R.I., Izatnagar, elicited reaction to tuberculin and was negative to johnin. Sheep was suspected for tuberculosis and subjected to s.c. tuberculin test. Significant rise in temperature of 3.5° F to 4° F was noted at 12–15 h after s/c test. Sheep was sacrificed and on post-mortem examination 3–4 abscesses of 1′ to 3′ diameter were found in the spleen. Abscesses were like that of goat.

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