Experimental allergic encephalomyelitis (EAE) in normal and T-lymphocyte deficient rats

Rats injected with encephalitogenic vaccine	No. of rats	No. of rats undergoing paralysis within days				Total paralyzed rats	Dead/ paralyzed rats
		8–14	15–21	22–28	29–35		
Normal	16	4	1		1	6/37%/	2/6
Thymectomized	10	1		1		2/20%/	1/2
Sham-thymectomized, irradiated, bone marrow reconstituted	11	5	2	1		8/72%/	6/8
Thymectomized, irradiated, bone marrow reconstituted	16	4	4	1	1	10/63%/	6/10

diseases. To obtain more information on mechanisms of EAE pathogenesis, we tried to induce the disease in T-lymphocyte deficient rats.

Materials and methods. Rats of WVM strain (derived from Wistar stock) of both sexes were used. They were thymectomized at the age of about 4 weeks. Through an incision in the neck, the thymus was sucked out of the thorax. 2 months after thymectomy, the rats were irradiated with a dose of 750 R. Irradiation constants were: 220 kV, 15 mA, filters 0.5 mm Cu and 1.0 mm Al, distance to target 40 cm, dose rate 100 r/56 sec measured in air. Within 4 h after irradiation, the rats were given i.v. 60×10^6 syngeneic bone marrow cells suspended in 1 ml of buffered physiological saline. 2 months after irradiation and bone marrow reconstitution, the animals were given intradermally 10 doses of 0.1 ml of the rat brain homogenate in complete Freund adjuvant mixture (Difco). Control groups consisted of sham-thymectomized, irradiated and bone marrow reconstituted rats, only thymectomized rats and normal rats. All controls, as also the experimental group, were given the encephalitogenic vaccine.

Results. As shown in the Table, irradiated rats either thymectomized or sham-thymectomized before irradiation and bone marrow reconstitution, showed a higher incidence of paralysis after the encephalitogenic vaccine injections than normal and thymectomized non-irradiated controls. This seems to suggest that irradiation favours the incidence and development of EAE. Thymectomy alone exerted a slight lowering of the EAE incidence in comparison with normal controls. Severity of the disease, as estimated after the mortality in sick rats, seems to follow the incidence of EAE.

Inspection of the brain and medulla sections stained with haematoxylin and eosin revealed mononuclear cell infiltrations and extravasates only in the group of normal control rats which fell ill after the inoculation of the encephalitogenic vaccine. In the paralyzed animals of all other groups, no cellular infiltration was observed.

Discussion. The enhancing effect of irradiation upon the development of EAE was expected in view of the earlier findings of a favorable activity of irradiation upon the incidence of the disease[4]. However, this effect of irradiation was also visible in the rats made deficient in T-lymphocytes, thus supporting the reports mentioned in the introduction that there is no essential need for a mononuclear cellular immune reaction to induce EAE[2]. This appeared to be further supported when no cellular infiltration could be found in the brain and medulla of T-lymphocyte deficient, severely paralysed rats.

The conclusion that B-lymphocytes had to be primed with the encephalitogenic vaccine in thymectomized, irradiated bone marrow recipients in the absence of T-lymphocytes appears erroneous. One possible explanation could also be based upon the assumption that the immunocytes transferred with the bone marrow were already primed with selfneural tissue antigens in normal bone marrow donors which had an intact thymus. The injection of the vaccine would act accordingly, as the booster antigen injection.

It is questionable if the lower sensitivity to the encephalitogenic vaccine in thymectomized nonirradiated rats could be ascribed to the lack of priming due to the T-lymphocyte deficiency after the thymus removal in young adult animals.

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Inflammatory Factors Produced by Sensitized Guinea-Pig Peripheral Blood Lymphocytes

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Summary. The supernatants obtained from stimulated tuberculin-sensitive guinea-pig peripheral blood lymphocytes contain factors that induce a cutaneous inflammatory response in normal guinea-pigs similar to the tuberculin reaction and inhibit the migration of normal guinea-pigs peritoneal exudate cells. There appears to be a correlation between the presence of in vitro migration inhibitory activity and inflammatory activity in vivo.

The production of soluble mediators such as migration inhibition factor (MIF) or skin reactive factor (SRF) has been demonstrated in the guinea-pig using lymph node cells[2,3] or peritoneal exudate lymphocytes[4,5] stimulated with antigens such as PPD or BGG.

Since it is generally accepted that the production of these mediators is an in vitro correlate of delayed hypersensitivity[6] it would be expected that circulating mononuclear cells i.e. those found in the peripheral blood, would be involved in cutaneous delayed hypersensitivity responses. In addition the in vivo assay of these mediators (as SRF) should correlate with in vitro results as assessed

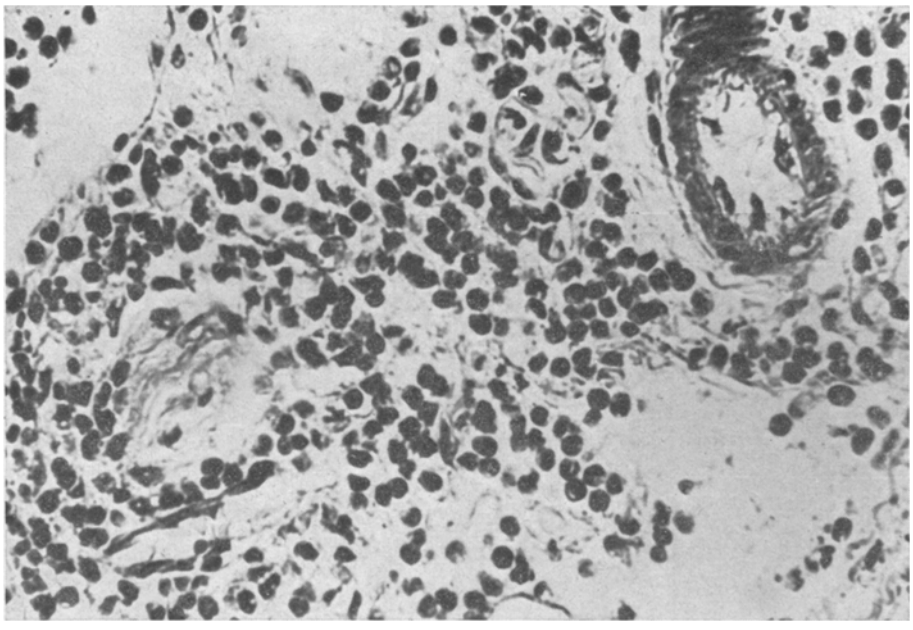

Fig. 1. The inflammatory infiltrate induced in normal guinea-pig skin by the injection of supernatants from PPD-stimulated tuberculin-sensitive guinea-pig peripheral blood lymphocyte cultures. H. & E. × 200.

in the MIF assay. For these reasons the production of soluble mediators by guinea-pig peripheral blood lymphocytes sensitized to tuberculin was examined.

Materials and Methods. Peripheral blood lymphocytes were obtained from BCG sensitized (without Freund's adjuvant) Hartley strain guinea-pigs by cardiac puncture and separation on a Ficoll/Hypaque gradient[7]. The degree of sensitivity of the animals as assessed by prior tuberculin testing was variable. Inflammatory supernatants were produced by culturing the mononuclear cells at a concentration of $1.0–3.0 \times 10^6$ cells/ml with PPD 2.5 μg/ml for 3 days in Medium 199 without serum. Supernatants were clarified by centrifugation at 16,000 × *g* for 20 min and PPD was added to control supernatants to give a concentration of 2.5 μg/ml. They were then dialyzed against 0.15 *M* NaCl for 24 h and against distilled water for 24 h. After filtration through 0.22 μm cellulose filters, the supernatants were lyophilized and stored.

Soluble mediators were assayed by (a) the intradermal injection into normal unimmunized guinea-pigs of the dialyzed lyophilized supernatants dissolved in Medium 199 to give a final concentration 20–50 times the original. Inflammatory reactions were assessed by measurement of erythema, induration and increase in skin thickness and by biopsy, skin sections being fixed with formaldehyde and stained with hematoxylin and eosin. (b) Macrophage migration inhibition using the dialyzed lyophilized supernatants dissolved in Medium 199 and 20% heat inactivated foetal calf serum to give a final concentration of 5–7 times the original. These were assayed on normal guinea-pig peritoneal exudate cells by the method of David et al.[8].

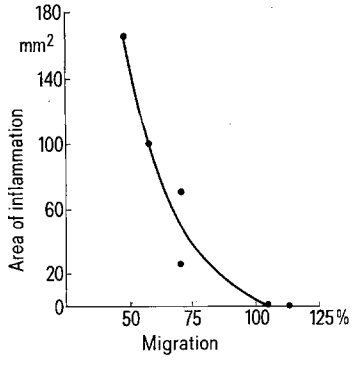

Fig. 2. The relationship between the migration inhibition factor activity and skin reactive factor in the same supernatants. MIF estimations were carried out in duplicate (variation between duplicates was < 10%) and the mean is shown, whilst SRF was assayed as a single intradermal injection. Each point represents assay results with supernatants from a single animal.

Results and discussion. The intradermal injection of supernatants from sensitized guinea-pig peripheral blood lymphocytes cultured with PPD produced an inflammatory response characterized by erythema and induration, reaching a maximum at 4–6 h and containing microscopically an infiltration of the deeper layers of the dermis with predominantly mononuclear cells (Figure 1).

At control injection sites, no inflammation and very scanty infiltration with cells was seen. The inflammatory response induced by the active supernatants was histologically similar to the tuberculin reaction but accelerated in its rate of development[9].

In addition, the presence of MIF was detectable in vitro, with inhibition of macrophage migration being found to occur in those supernatants producing an

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inflammatory response in vivo (Figure 2). There appears to be a good correlation between the presence of SRF and MIF in the same supernatant. This does not however indicate that MIF is solely responsible for the inflammatory reaction produced, since the culture supernatants will contain a number of different soluble factors.

Thus peripheral blood mononuclear cells, the cells that are presumably initially involved in the tuberculin reaction have been shown to produce an inflammatory factor and a factor inhibiting the migration of macrophages.

The production of migration inhibition factor in serum-free medium using cells from BCG-immunized guinea-pigs makes it unlikely that cytophilic antibodies are involved [10]. In addition, the lack of significant polymorphonuclear leukocyte infiltration in the supernatant induced skin reaction is against the involvement of antigen-antibody complexes.

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Localisation de dioctyl-phtalate sur les immunoglobulines G normales et sur celles de la maladie de Kahler

Localization of Dioctyl-Phthalate on Normal Immunoglobulins G and on those of Kahler's Disease

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Summary. After having identified as dioctyl-phthalate (DOP), a plasticizer that comes out with fatty acids of normal immunoglobulins G (IgG) and of those of Kahler's disease, we found out that DOP is preferentially bound to the heavy chains of the IgG and is either missing from myeloma proteins or more abundant in them than in normal ones.

Le dioctyl-phtalate (DOP) est l'un des esters de l'acide phtalique utilisé comme plastifiant du chlorure de polyvinyle (PVC). Lors des plasmaphérèses, des poches en matière plastique sont utilisées dans le but de conserver le sérum prélevé. A l'occasion de travaux antérieurs, en analysant les acides gras (AG) des immunoglobulines G (IgG)[2], des immunoglobulines A[3] et des fragments papaïniques des IgG[4] de malades atteints de la maladie de Kahler, nous avons mis en évidence par chromatographie en phase gazeuse et identifié par spectrométrie de masse, un composé à temps de rétention élevé qui s'est avéré être du dioctyl-phtalate[4]. Nous avons pu établir que ce phtalate provenait principalement d'échantillons de sérums ayant été contenus dans du matériel en matière plastique. Nous exposons ici comment nous avons pu déterminer une localisation du dioctyl-phtalate sur les IgG normales et myélomateuses.

Matériel et méthodes. Nous avons obtenu deux types de sérums d'une part en mélangeant des sérums normaux ne présentant aucune anomalie électrophorétique et d'autre part en isolant des sérums pathologiques par plasmaphérèse. Ces sérums sont conservés à −24 °C dans des récipients en matière plastique. Parallèlement un lot de sérums a été maintenu dans les mêmes conditions dans des «vacutainers» en verre. A partir des différents sérums les protéines sont isolées par chromatographie sur résines échangeuses d'ions[5]. L'hydrolyse papaïnique est controlée par immunoélectrophorèse. Les fragments Fab et Fc de l'IgG sont ensuite purifiés par électrophorèse de zone[4] et par chromatographie d'affinité[6]. On vérifie la pureté des fragments par immunoélectrophorèse. Par chromatographie sur couche mince, après extraction lipidique[7] les acides gras libres ainsi que les triglycérides et les esters du cholestérol sont recueillis. Ces deux derniers sont saponifiés ce qui permet de séparer leurs acides gras. Les acides gras libres sont méthylés. Tous les acides gras sont alors analysés par chromatographie en phase gazeuse sur appareil Varian 1400 à ionisation de flamme. Leur identification s'effectue à l'aide de témoins analysés dans les mêmes conditions. Le DOP qui apparaît parmi les acides gras est authentifié par un étalon interne de

Tableau I. Teneurs en DOP de protéines normales et myélomateuses provenant de récipients en matière plastique; d'un sérum normal conservé dans du matériel en verre

	AGL	AG extraits des TG	AG extraits des EC	AG totaux
IgG normale	—	28	11	13
IgG myélomateuse (Malade Sen...)	—	45,77	—	15
IgG myélomateuse (Malade Cap...)	28,5	18,7	12,9	20
Sérum normal	1,1	1,3	10,9	4,4

Les pourcentages de DOP sont déterminés par le calcul automatique, à l'aide d'un intégrateur Infotronics, des surfaces comparées des tracés caractéristiques des acides gras et du DOP sur les courbes chromatographiques. AGL, Acides gras libres; TG, triglycérides; EC, esters de cholestérol.

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