1 Supported by the Armed Forces Radiobiology Research Institute, Defense Nuclear Agency, under Research Work Unit MJ00018. Views presented in this paper are those of the authors; no endorsement by the Defense Nuclear Agency has been given or should be inferred.
2 Research was conducted according to the principles enunciated in the 'Guide for the Care and Use of Laboratory Animals' prepared by the Institute of Laboratory Research, National Research Council.
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Production of the lymphokine, macrophage aggregating factor, is not inhibited by histamine

P. Badenoch-Jones

Department of Experimental Pathology, John Curtin School of Medical Research, Australian National University, Canberra, A.C.T. 2602 (Australia), 23 April 1982

Summary. Unlike the previously reported inhibitory effect of histamine on the production of the lymphokine, migration inhibitory factor, histamine did not inhibit the production of macrophage aggregating factor (MAgF). By contrast, both prostaglandin E_2 and hydrocortisone inhibited MAgF production, in a dose-dependant manner.

Histamine has previously been shown to inhibit delayed hypersensitivity reactions in vivo[1,2], and lymphocyte proliferation and production of migration inhibitory lymphokines in vitro[3,4]. Inhibition of migration inhibitory factor (MIF) production has been shown to be mediated through a histamine-induced suppresor factor of mol.wt 23,000-40,000, produced by lymphocytes with H_2 receptors[3,5]. It has thus been postulated that histamine acts as a negative modulator of cell-mediated immune reactions. MAgF is a lymphokine activity which rapidly increases the adherence of macrophages to each other and to inert surfaces[6]; its relationship to MIF is not known. The production of MAgF has been shown to be immunologically specific and to be well correlated with the state of delayed hypersensitivity, in the guinea-pig[7]. It is shown here that MAgF production is not inhibited, indeed is slightly enhanced, by histamine. By contrast, 2 other agents reportedly inhibiting the production of MIF activity, prostaglandin E_2 (PGE_2) and hydrocortisone (HC), also inhibited MAgF production.

Methods. MAgF was produced by culturing 10×10^6 viable spleen cells/ml, from guinea-pigs immunized 14 days previously with 2 mg BCG (Bacillus Calmette Guerin) vaccine, with 50 µg/ml PPD (purified protein derivative) for 24 h in serum-free Eagles medium, as previously described[8]. Control cultures were reconstituted with PPD at the end of the culture period. Culture supernatants were dialyzed extensively against tap water and insoluble protein removed by centrifugation. After reconstitution of supernatants with 0.1 vol. of 10×Eagles medium, samples were assayed for MAgF activity. Oil-induced guinea-pig peritoneal exudate cells (containing > 80% macrophages) were prepared and their aggregation quantitatively measured, as previously described[8]. Histamine and hydrocortisone Na-succinate (both from Sigma Chemical Co.) were added to spleen cell cultures as saline solutions, and PGE_2 (Sigma Chemical

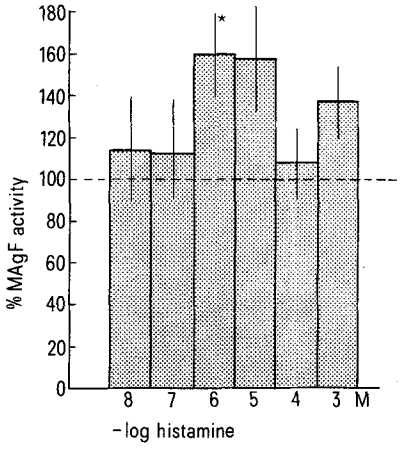

Figure 1. The effect of added histamine on the production of macrophage aggregating factor. Spleen cells, from BCG immunized guinea-pigs, were cultured with PPD antigen in the presence and absence of histamine. Dialyzed culture supernatants were then assayed for aggregating activity. Results are mean ± SEM for 3-6 separate cultures. * $p < 0.05$.

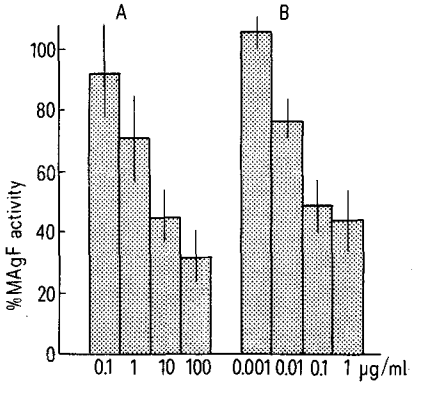

Figure 2. The effects of added hydrocortisone 21-succinate (A) and prostaglandin E_2 (B) on production of macrophage aggregating factor by guinea-pig spleen cells.

Co.) as an ethanolic solution. The final ethanol concentration did not exceed 0.5 µl/ml and ethanol controls were also set up.

Results and discussion. As shown in figure 1, histamine did not inhibit MAgF production, over a wide concentration range. Indeed, there was a strong tendency for histamine to enhance MAgF production; although this was only marginally significant at 10^{-6}M ($p < 0.05$). By contrast, as shown in figure 2, both PGE_2 and hydrocortisone gave a dose-dependant inhibition. Previously, PGE_2 (0.1–1.0 µg/ml) has been shown to inhibit MIF production[9,10] although it enhances the production of 2 other lymphokines, skin reactive factor[10] and osteoclast activating factor[11]. Thus, PGE_2 may either enhance or depress the production of lymphokines, depending on which activity is measured. HC has been shown to either inhibit[12] or not inhibit[13] guinea-pig MIF production. Additionally, HC has been claimed to inhibit MAgF production[14], although a subjective assay system was used in this study. Histamine, unlike HC and PGE_2 which act directly on lymphokine-producing lymphocytes, inhibits MIF production indirectly through H_2 containing supressor lymphocytes. These cells are present in immunized and non-immunized guinea-pig spleen cell populations[5] and so the lack of inhibitory effect found in the present study should not be due to lack of this cell population. In conclusion, the inhibitory effects of histamine on lymphokine production have, to date, been shown only for migration inhibitory activities. The results presented here suggest that this is not generally applicable to all lymphokines.

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Enhancement of macrophage colony-stimulating factor in mice by carbon particles[1]

M. Tsurusawa, H. Izumi, J. Fujita and K.J. Mori

Department of Microbiology, Faculty of Medicine, Kyoto University, Kyoto 606 (Japan), 2 March 1982

Summary. Carbon particles enhance hemopoiesis in irradiated mice. Serum from carbon-treated mice stimulated macrophage colony formation, and inhibited granulocyte colony formation. The finding suggests that carbon-treatment modulates the hemopoietic environment through the monocyte-macrophage system.

Colony-stimulating factor (CSF) stimulates the formation of granulocyte-macrophage colonies from granulocyte-macrophage progenitors (GM-CFC)[2,3]. An elevated serum CSF level was reported in response to neutropenia[4]. Injection of bacterial lipopolysaccharide (LPS) into mice induces increases in serum CSF levels[5], granulopoiesis[6], and in the survival of irradiated animals[7].

Blockade of the reticuloendothelial system (RES) by particulate substances such as carbon and latex particles also stimulates the recovery of hemopoiesis in irradiated mice[8,9]. Since the major source of CSF appears to be the monocyte-macrophage system in RES[10], the enhancement of hemopoietic recovery by RES-blockade in irradiated mice may also be due to the elevated serum CSF levels.

We found that the injection of carbon particles into mice enhanced serum CSF, thereby stimulating macrophage production.

Materials and methods. Mice of strain DDY were used. Serum was collected from the mice 3 and 24 h after the injection of carbon particles (Pelikan ink, Germany). Bone marrow cells were incubated in a petri dish (10^5 cells/dish) containing 0.3% agar, 25% horse serum in Fischer's medium and 15% mouse lung- and heart-conditioned medium as a source of standard GM-CSF[3]. Test serum for CSF assay was added to the soft-agar culture instead of CSF. After 7 days incubation at 37 °C in 5% CO_2 in air, colonies consisting of 50 or more cells were counted, and CSF level was expressed as the number of colonies per 10^5 bone marrow cells.

Results and discussion. Injection of more than 1 mg carbon particles protected mice from the lethal effects of irradiation, but 0.1 mg carbon particles did not protect the mice effectively (table 1). All doses of carbon particles tested, however, increased serum CSF levels 3 h after injection. The activity disappeared within 24 h after carbon treatment. Thus, there was a discrepancy between the dose of carbon particles for enhancement of serum CSF level and that for radioprotection.

All the colonies consisted of macrophages, and no granulocyte or granulocyte-macrophage (mixed) colonies were found in the culture. To determine whether this is due to the low concentration of serum tested, soft-agar culture was performed with various concentrations of the serum. The number of colonies per 10^5 marrow cells increased, depending on the amount of test serum. However, all the colonies thus induced were again macrophage colonies, wereas serum from LPS-treated mice induced all types of colonies (table 2). To determine whether or not the serum from carbon-treated mice actually inhibits the granulocyte colony formation, serum from carbon-treated mice was added to the bone marrow cell cultures that were stimulated with GM-CSF. Addition of 5% carbon-serum did not affect the ratio of colony types (data not shown), but the addition of 20% carbon-serum inhibited granulocyte colony formation completely (table 2).

It has been convincingly shown that murine CSF preparations contain different CSFs: one that primarily stimulates macrophage production and another that simulates granu-