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NON-SPECIFIC MIF-LIKE ACTIVITY INDUCED BY THE SYNTHETIC IMMUNOADJUVANT : N-ACETYL MURAMYL-L-ALANYL-D-ISOGLUTAMINE (MDP)

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SUMMARY

N-acetyl-muramyl-L-alanyl-D-isoglutamine (MDP) which represents the active part of mycobacteria for adjuvanticity is able to inhibit the migration of peritoneal exudate cells. This inhibitory action of MDP is not due to cytotoxicity because colchicine which has been shown to inhibit MIF also restores the migratory properties of MDP treated cells, and pretreatment of peritoneal cells with MDP has no influence on their later migration. There could be a relation between structural requirements for adjuvant activity and macrophage migration inhibition because the D-D-stereoisomer of MDP which is inactive as an adjuvant, has no inhibitory properties.

INTRODUCTION

N-acetyl-muramyl-L-alanyl-D-isoglutamine, a constituent of most bacterial cell wall peptidoglycans, is the minimal structure which can substitute for mycobacteria in FCA (1-3). It was shown that MDP is adjuvant active in rats (4) or mice even in absence of paraffin oil either *in vivo* (5) or *in vitro* (6, 7, 8). We have previously shown that addition of MDP to an emulsion of soluble antigen in FIA leads to a delayed hypersensitivity with specific release of MIF by sensitized lymphocytes in the presence of antigen (9). Besides this specific release of MIF by sensitized lymphoid cells(10, 11),MIF like-compounds have been obtained from supernatants of mixed lymphocyte cultures or from cultures stimulated by non specific mitogens such as Con A or periodate (12). MDP, in usual culture conditions has no mitogenic properties, when cultured in medium containing fetal calf serum (13).

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We have observed that MDP is able to inhibit the migration of peritoneal exudate cells (PE cells) from guinea pigs sensitized to ovalbumin 3 months before, although at this time these cells did not produce any more specific MIF. The PE cells from normal guinea pigs were also inhibited by MDP but for a still unexplained reason, 20% of animals were unsensitive to MDP.

While these results were submitted for communication (14) similar results have been reported by Yamamoto et al. (15).

MATERIAL AND METHODS

Reagents :

The synthetic adjuvant MDP and analogues were kindly provided by Dr. Lefrancier from Choay laboratories, Montrouge, France.

Peritoneal exudate cells :

PE cells were obtained as reported previously (9) : Hartley female guinea pigs weighing 500 g were injected intraperitoneally with 10 ml of sterile paraffin oil, 4 days later peritoneal cells were collected with 50 ml of Hanks balanced salt solution containing 5 U of heparin per ml, 100 U of penicillin plus 100 µg of streptomycin ; after 3 washings by centrifugation at 200 g, cells were suspended in RPMI 1640 containing antibiotics and 12% fetal calf serum (Gibco Bio Cult, Glasgow) to a concentration of 50×10^6 cells per ml.

Migration tests were performed as reported previously (9) in capillary tubes placed in Mackaness-type microchambers (sterilin plates) filled with the same medium containing or not synthetic compounds ; each assay was performed in six replicates. Migrations were measured by projection and planimetry Migration indexes were calculated as follows :

$$MI = \frac{\text{mean area of migration of six capillaries in antigen, adjuvant or analogue containing medium}}{\text{mean area of migration of six capillaries in medium alone.}}$$

Results for a typical experiment are reported.

Adherent cells :

PE cells were incubated (10^7 cells per ml of RPMI containing antibiotics and serum) for 2 hours at 37° ; supernatant cells were removed

Table I : Migration indexes of peritoneal exudate cells from guinea pigs previously sensitized to ovalbumin.

Migration index = $\frac{\text{mean of migration of areas with medium + ovalbumin or MDP}}{\text{mean of migration of areas with medium alone}}$

Each test is performed on 6 replicates

Results of a typical experiment (out of six)

	3 weeks after sensitization		4 months after sensitization			
	ovalbumin		ovalbumin		MDP	
Guinea pigs sensitized to ovalbumin	0	100µg/ml	0	100µg/ml	1 µg	10 µg
in FIA	1	0.72	1	1.06	0.16	0.22
in FIA + MDP	1	0.24	1	0.95	0.23	0.27

and after vigorous washings adherent cells were recovered with a rubber policeman.

RESULTS

Action of MDP on the migration of PE cells from immunized animals.

In a first set of experiments PE cells were obtained from guinea pigs sensitized to ovalbumin, either in Freund's incomplete adjuvant (Difco) or in alginate (Technam) 4 months before. Results of a typical experiment out of six are given in Table I. As already reported, PE cells from MDP injected guinea pigs, 3 weeks after immunization, produce specific MIF (9) ; 4 months later, the addition of antigen is no more able to release MIF from the lymphoïd cells: however addition of MDP to the medium greatly inhibits the migration of macrophages.

Action of MDP on the migration of PE cells from normal animals

The following experiments were realized with PE cells from normal animals. In 2 out of 10 guinea pigs, PE cells migration was not sensitive to MDP. Table II shows that a dose response is obtained and the maximum of the inhibition occurs with less than 1 µg/ml. Colchicine has been shown to enhance the migration of normal macrophages and to protect them from the action of MIF (16). We have observed similar results and in every case a complete inhibition of MDP action in 10^{-6}M colchicine.

Table II : Migration indexes of peritoneal exudate cells - from guinea pigs. Influence of Colchicine, pretreatment with MDP and removal of non adherent cells on the inhibitory effect of MDP.

	MDP in µg/ml					
	0	0.1	0.5	1	2.5	5
Medium alone	1	0.36	0.23	0.28	0.37	0.41
Medium + 10^{-6}M Colchicine	1	1.16	1.09	1.08	1.12	1.23
Pretreatment with MDP	1	-	-	0.32	-	-
After removal of non adherent cells	1	-	-	0.36	-	-

Table III : Effect of MDP and analogues on the immune response and migration of peritoneal cells - Migration indexes. The adjuvant properties of the compounds tested are recalled in the first columns.

	Adjuvanticity in					
µg/ml	guinea pigs	mice	0	1	5	10
Mur Nac-L-Ala-D-iso-Gln (MDP)	+	+	1	0.22	0.27	0.32
Mur Nac-D-Ala-D-iso-Gln (DD)	-	-	1	1.07	0.98	0.89
Mur Nac-L-Ala-D-Glu-OH	±	+	1	0.28	0.36	0.38
MDP + DD (5 mg)			1	0.24		

This inhibition is not due to a toxic effect of MDP because, viability of PE cells is not affected (measured by 1‰ trypan blue exclusion). Furthermore, PE cells have been incubated for 2 hours at 37° with MDP (1 µg/ml). The recovered cells have the same migration index as original cells and are still sensitive to the inhibitory effect of MDP. Table II also shows that the migration of adherent cells is inhibited by MDP, suggesting a direct action on macrophages rather than a release of MIF.

Effect of analogues of MDP

We have compared the effect on migration of 2 analogues : N-acetyl-muramyl-L-alanyl-D-glutamine (which is active as an adjuvant only on the secondary response in guinea pigs (9) but active in the mice in saline (17)) and the D-D-stereoisomer N-acetyl-muramyl-D-alanyl-D-isoglutamine (inactive as an adjuvant). Table III shows that the two active compounds inhibit the migration of macrophages but the D-D analogue inactive as an adjuvant, has no action on the migration. This D-D analogue of MDP which was shown previously (18) to have antiadjuvant properties when injected with an antigen in FIA is without effect on the inhibitory property of MDP on the migration of peritoneal cells (Table III).

DISCUSSION

N-acetyl-muramyl-L-alanyl-D-isoglutamine was first described as a substitute for mycobacteria in Freund's adjuvant (1-3),its adjuvant properties have also been established in saline *in vivo* or *in vitro*(5, 6, 7, 8)

The mechanism of action of MDPis now investigated in several laboratories. A mediation by T lymphocytes was suggested by the experiments of Löwy *et al.* (19) on the response of irradiated and reconstituted mice to sheep red blood cells. Results of Prunet *et al.* (20) on the restoration of a specific immune response in mice depleted of antibody forming cells, also show an action of MDP on T cells. An activation of macrophages *in vitro* was demonstrated by Juy *et al* (21) and Février *et al* (22) have shown that the stimulation of the immune response *in vitro* is mediated by a factor released by macrophages. Recently Watson *et al.* (23) could show that MDP can replace the helper function of T cells in the primary immune response of T cells depleted spleen cultures ; probably

by acting directly on B precursor cells. These different results show that MDP can activate several classes of cells, may be directly or by the mediation of factors released from the initial target cell.

We describe here a non specific inhibition of the migration of peritoneal cells by MDP. This inhibitory activity has been observed as well on total peritoneal cells as on purified adherent cells, suggesting rather a direct effect of MDP on macrophages than a release of MIF. The reversibility of the inhibition is against an action of MDP due to any cytotoxic effect and this is confirmed by the complete inhibition of MDP action by 10^{-6} M colchicine.

Recently and independently from our results Yamamoto et al. (15) have reported too the inhibition of macrophages migration by MDP and they have good arguments for a direct action of MDP on macrophages ; their dose effect is different from ours : inhibition increases with increasing doses of MDP (till 100 µg) in our experiments the optimal dose for a complete inhibition is about 1µg ; they report the non activity of the L-L analogue of MDP, we have observed the inactivity of the D-D isomer. In preliminary experiments, analogues of MDP previously prepared to define the adjuvant active structure (18) have been tested ; results obtained with three adjuvant active compounds (resulting from the substitution in the N-acetylmuramyl-L-alanine-D-isoglutamine of L-Ala by L-**Ser** , Mur-N-acetyl by Mur-N-butyryl or D-GluαNH_2, γOH by D-GluαNH_2, $\gamma O CH_3$) and three inactive analogues (muramicitol-L-Ala-D-isoGln, Lactyl-L-Ala-D-isoGln and MurNac-L-Ala-D-isoAsn) show a good relation between adjuvant activity and ability to inhibit the migration of peritoneal cells. These results suggest similar structural requirements for adjuvant activity and inhibition of macrophages migration.

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