Effect of N-Acetyl-muramyl-L-alanyl-D-isoglutamine on Interferon Production in Mice by Newcastle Disease Virus

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SUMMARY

The activity (carbon clearance) of the reticuloendothelial system (RES) of mice inoculated intraperitoneally with N-acetyl-muramyl-L-alanyl-D-isoglutamine (muramyl dipeptide, MDP) was greatly stimulated 1 day, but not 7 days after MDP treatment. No enhancement of resistance to ectromelia virus infection and influenza virus infection in mice treated with MDP was observed. In mice splenectomized 1 week after MDP pretreatment, normal levels of circulating interferon were produced in response to Newcastle disease virus (NDV), whereas in the mice treated with MDP after splenectomy, circulating interferon levels were reduced to the same level as produced in the MDP-untreated and splenectomized mice. Interferon production in response to NDV was augmented in non-adherent peritoneal and spleen cell cultures derived from MDP-pretreated mice, whereas it was reduced in peritoneal and splenic macrophage cultures. These results suggest that the non-adherent spleen cells activated with MDP were disseminated from the spleen to other organs, that the lack of enhancement of interferon production in mice pretreated with MDP might be due to reduced interferon production in macrophages, and that the activation of the RES of the whole body by MDP did not correlate with the enhancement of interferon production in spleen cells or with the reduction of interferon production in macrophages.

Several kinds of agents which have an adjuvant activity have been shown to induce non-specific resistance to viral infection (Gledhill & Rees, 1960; Gorhe, 1967; Floc’h & Werner, 1976; Starr et al., 1976; Lodmell & Ewalt, 1978; Suenaga et al., 1978). In a previous report (Sakuma et al., 1983), we have shown that mice inoculated with heat-inactivated BCG acquired a high non-specific resistance to ectromelia virus infection, and that this acquired resistance could be mainly ascribed to enhanced interferon production in the spleen of BCG-inoculated mice in response to the virus. N-acetyl-muramyl-L-alanyl-D-isoglutamine (muramyl dipeptide, MDP) is the minimal active portion of peptidoglycan of the bacterial cell wall required for an adjuvant activity by intramuscular, intravenous or oral routes of administration (Ellouz et al., 1974; Kotani et al., 1975; Merser et al., 1975; Adam et al., 1976; Kusumoto et al., 1976; Ohkuni et al., 1979; Tanaka et al., 1977, 1979). The purpose of the present study was to determine whether MDP had the same function as heat-inactivated BCG (H-BCG), augmenting a non-specific host resistance to viral infection by enhanced interferon production.

Eight- to 12-week-old female DDN mice were obtained from the closed colony of the Institute for Experimental Animals of our college. Splenectomy was carried out as described previously (Sakuma et al., 1983). The Miyadera strain of Newcastle disease virus (NDV) used as an interferon inducer was prepared as in the preceding report (Sakuma et al., 1983). Resident peritoneal cells were collected from normal mice and MDP-treated mice by washing the peritoneal cavity with Eagle’s minimum essential medium supplemented with 10% newborn calf serum (MEM-NCS10). Spleen cells were collected by straining minced spleen tissues through a stainless steel mesh. These cells were incubated in a Roux bottle at 37 °C for 24 h, the non-adherent cells were collected by pipetting, and the adherent cells were collected by scraping the glass wall of the bottle with a rubber policeman. These peritoneal cells, spleen cells and L-929
Fig. 1. Effect of MDP and splenectomy on carbon clearance in mice. Mice treated with 200 μg MDP and/or splenectomized were intravenously injected with colloidal carbon suspension ('Pelikan' special ink C11/1431a, Günther Wagner, Hanover, F.R.G.) at a rate of 0.16 mg/0.01 ml/g of mouse body weight. The amount of carbon retained in the serial blood samples was measured by absorbance at 610 nm. ○, Untreated; ■, ▲, MDP-treated mice after 1 day or 7 days respectively; □, Δ, MDP-treated mice splenectomized 1 day or 7 days after treatment respectively.

cells were cultivated in MEM-NCS10, and maintained with MEM-NCS5 after induction with NDV. MDP was obtained from Groupement d'Intérêt Economique Institut pour la Recherche et la Production d'Immunostimulants (G.I.R.P.I.), Paris. Methods for sampling and assaying the mouse blood for interferon, use of the mouse reference standard interferon (reagent number S-002-904-511), and measurement of the carbon clearance activity of the reticuloendothelial system (RES) of mice have been described in a previous paper (Sakuma et al., 1983).

Interferon-inducing activity of MDP was investigated in mice inoculated intravenously and intraperitoneally with 10 to 200 μg MDP, and also in adherent spleen cell, non-adherent spleen cell, adherent peritoneal cell, non-adherent peritoneal cell and L-929 cell cultures induced with 1 to 100 μg per ml of MDP. MDP had no interferon-inducing activity in mouse or cell cultures (interferon titres of all samples were less than 10 IU).

Carbon clearance activity of mice was measured 1, 7 and 28 days after intraperitoneal inoculation of 200 μg MDP. As shown in Fig. 1, the activity was greatly enhanced in mice 1 day after treatment with MDP. However, the activity had returned to the control value 7 days (and 28 days, data not shown) after treatment. The enhancement of RES activity in mice 1 day after treatment with MDP was not affected by splenectomy after MDP treatment, as shown in BCG- or H-BCG-treated mice (Sakuma et al., 1983). The mice treated intraperitoneally with 200 μg MDP (hereafter designated as MDP-treated mice) were inoculated intraperitoneally with 10² LD₅₀ of ectromelia virus (Ishibashi strain) or intranasally with 10² LD₅₀ of influenza virus (A/PR/8/34) 1 day and 7 days after MDP treatment. No enhancement of resistance to virus infection in mice treated with MDP was observed (data not shown). Early reports have shown that MDP stimulates the resistance of animals against bacterial and mycotic infection (Chedid et al., 1977; Cummings et al., 1980; Matsumoto et al., 1979, 1981; Yapo et al., 1982). The present results suggest that the augmentation of activity of the RES by MDP treatment does not induce resistance to viral infection, which is thus different from bacterial and mycotic infection, and that spleen cells do not participate in the augmentation of RES activity by MDP treatment.
Circulating interferon production in the MDP-treated mice in response to NDV was investigated. The results presented in Fig. 2(a) show that circulating interferon production was not enhanced in mice treated with MDP either 1 day or 7 days before induction, and that interferon production did not correlate with the RES activity of the whole body. Then, in order to obtain information on the role of the spleen in circulating interferon production, the mice were treated with MDP, splenectomized 1 week later, and induced with NDV after a 1 week recovery period. These results are presented in Fig. 2(b). The splenectomized mice produced a markedly reduced interferon level, as shown in our previous paper (Sakuma et al., 1983), but mice treated with MDP before splenectomy produced a normal level of interferon. In marked contrast to the above results, Fig. 2(c) shows that mice treated with MDP after splenectomy produced less interferon. These results suggest that the spleen cells that have an interferon-producing ability in response to NDV were activated with MDP and that these cells disseminate from the spleen to other organs. But this does not explain the fact that circulating interferon production in mice treated with MDP was not enhanced (Fig. 2a). Experiments were then performed to determine whether MDP had a suppressive effect on interferon production by other cell species in response to NDV. Adherent peritoneal cells, non-adherent peritoneal cells, adherent spleen cells and non-adherent spleen cells derived from normal mice and MDP-treated mice were adjusted to 2 x 10^6 cells/ml, and inoculated with NDV at a multiplicity of infection of 10 p.f.u./cell. As shown in Fig. 3, interferon production in the adherent peritoneal cells and adherent splenic cells derived from the MDP-treated mice was greatly reduced. In contrast, interferon production in the non-adherent spleen cells derived from the MDP-treated mice was augmented. No difference in interferon production was observed between non-adherent peritoneal cells derived from normal mice and MDP-treated mice.

One of the reasons why MDP failed to enhance the resistance of mice to ectromelia virus and influenza virus infection might be due to reduced interferon production in macrophages of MDP-treated mice, although the mechanism of reduction of interferon production in the peritoneal and splenic macrophages is still unknown. One possibility for the mechanism of depression of interferon production may be the induction of refractoriness to the other inducer in the adherent cells by MDP. In the present study, interferon was not detected in adherent and non-adherent peritoneal cell cultures and spleen cell cultures induced in vitro with 1 to 100 µg per ml of MDP. These findings, however, do not rule out the possibility that MDP is responsible for
Fig. 3. Interferon production by *in vitro* cultures of peritoneal adherent cells (a), peritoneal non-adherent cells (b), splenic adherent cells (c), and splenic non-adherent cells (d) derived from MDP-treated or untreated mice. O, Cells of MDP-untreated mice; ●, △, cells of MDP-treated mice after 1 day or 7 days respectively.

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**REFERENCES**


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