Stimulation of Immune Response by Polymannuronic-Guluronic Acid in Mice

Natural and synthetic polynucleotides (PN) are known to stimulate immune response in mice immunized with sheep red blood cells (SRBC)[1]. We previously suggested that polyanions generally may enhance immune response, and that the stimulating activity of PN on immune response, may be related to their polyanionic character[2]. WACKER et al.[3] have reported that alginic acid, (polymannuronic-guluronic acid) also a polyanion, inhibits cell-free protein synthesis but stimulates approximately twofold immune response towards SRBC in vitro.

According to BRAUN et al.[1] single-stranded PN stimulate immune response in vitro but not in vivo. However, double-stranded PN enhance immune response in vivo. These differences may be due to the known fact that double-stranded PN are more resistent to enzymatic degradation than single-stranded PN. From these results one can suggest that polyanions which stimulate immune response in vitro do not necessarily enhance immune response in vivo.

In this report we describe the influence of polymannuronic-guluronic acid (pMG) on the primary immune response in mice. 6–8 week-old female NMRI/HAN mice were inoculated i.p. with SRBC suspended in 0.5 ml of 0.15 *M* phosphate buffered saline (PBS). pMG (alginic acid, purchased from C. ROTH, OHG, Karlsruhe) suspended in 0.5 ml of PBS was injected i.p. or i.v. 19S plaque-forming cells (PFCs) in the spleen were assayed according to L'AGE-STEHR and HERZENBERG[4] at times indicated after immunization of mice. Time course experiments (Figure 1) show that 4 mg pMG per mouse enhance PFC-response in mice inoculated with suboptimal SRBC doses (2×10^6 SRBC/mouse).

This effect is dependent on the pMG dose administered (Figure 2). 4 days after antigen inoculation a 44fold enhancement of immune response was observed with 4 mg pMG/mouse. A significant enhancement of PFCs was noticed even when 0.4 mg of pMG per mouse was injected.

Results in the Table clearly demonstrate that a direct contact of SRBC and pMG was not a requirement for the adjuvant action of this polyanion. The stimulation of PFC-response was more effective giving antigen and polyanion by the same route, but significant stimulation of

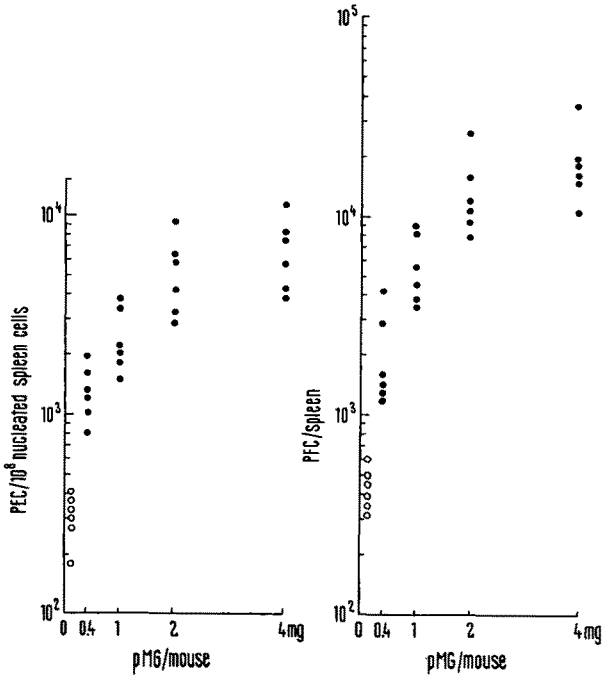

Fig. 2. The effect of various doses of polymannuronic-guluronic acid (pMG) on the number of antibody-forming spleen cells (plaque-forming cells) in mice. Groups of mice were injected with graded amounts of pMG i.p., 15 min after inoculation of mice with 2×10^6 sheep red blood cells. Plaque-forming cells (PFC) were assayed 4 days after antigen inoculation. (6 mice per group).

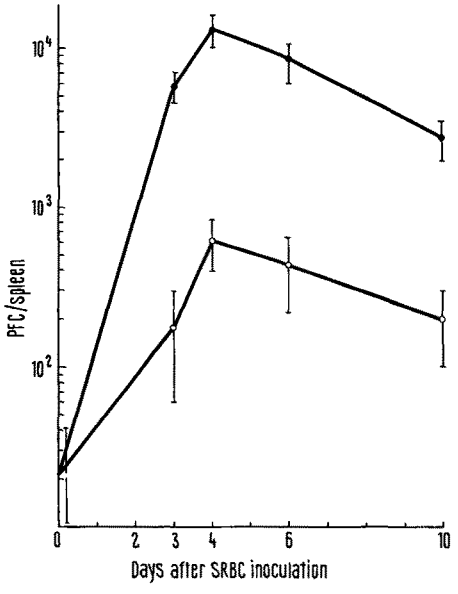

Fig. 1. Time course study of the effect of polymannuronic-guluronic acid (pMG) on PFC-response. Mice were injected with 4 mg pMG 15 min prior to SRBC inoculation (●). Controls (○) and pMG-treated animals were inoculated with 2×10^6 SRBC i.p. Each point represents average values of 6–8 mice. The standard errors are shown by the vertical bars.

Effect of route on adjuvant action of polymannuronic-guluronic acid (pMG)

Route of SRBC	Route of pMG	PFC/Spleen average	±S.E.
–	–	26	20
i.p. 2×10^6	–	520	160
i.p. 2×10^6	i.p. 4 mg	16,500	4,300
i.p. 2×10^6	i.v. 4 mg	11,600	2,400

Mice were injected with pMG 15 min prior to sheep red blood cell (SRBC) inoculations. Plaque-forming cells (PFC) were assayed 4 days after SRBC inoculation (6 mice per group).

[1] W. BRAUN, M. NAKANO, L. JARASKOVA, Y. YAJIMA and L. JIMINEZ, in *Nucleic Acids in Immunology*, (Eds. O. J. PLESCIA and W. BRAUN; Springer-Verlag Inc., New York 1968), p. 347.

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immune response took place also when antigen and polyanion were given by different routes.

JOHNSON et al.[5] have reported that polyriboadenylic-polyribouridylic acid enhances immune response only wehn it is given close to the time of antigen injection. According to Figure 3, under our experimental conditions,

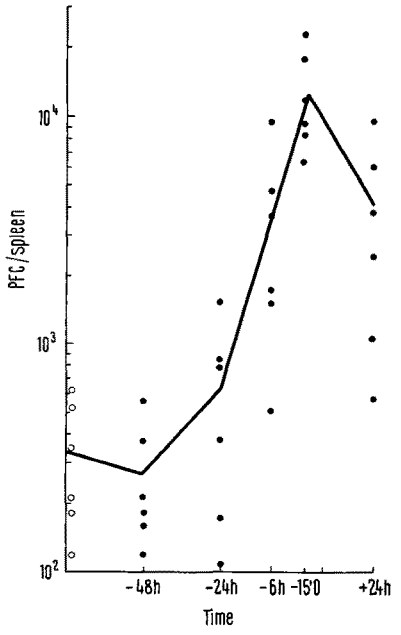

Fig. 3. The effect of timing on the adjuvant action of polymannuronic-guluronic acid (pMG). Groups of mice (6 mice per group) were injected with 4 mg pMG per mouse at various times prior or after antigen inoculation. Controls (○) and pMG-treated (●) mice were inoculated with 2×10^6 sheep red blood cells per mouse. Plaque-forming cells (PFC) were assayed 4 days after antigen inoculation.

using pMG instead of polyriboadenylic-polyribouridylic acid as adjuvant and SRBC instead of bovine-γ-globulin as antigen, similar but not identical results were observed. pMG enhances immune response, when given 6 h or 15 min prior to the antigen inoculation. However, pMG given 1 day after immunization causes significant enhancement of PFC-response. pMG injected 1 or 2 days before SRBC inoculation has no significant effect on PFC-response. It is of interest to note that pMG did not cause increase in spleen weight and erythropoesis in the spleen as observed with pyran copolymere, a carboxylate polymere[6] and polyacrylic acid[2], both known to stimulate immune response in mice towards SRBC. BRAUN et al.[1] discussed possible modes of action of polynucleotides on antibody formation. We recently[2] discussed possible modes of action of polyanions on immune response at cellular and at subcellular level, but experimental data available at present do not yet permit interpretation of the effects observed.

Zusammenfassung. Bei Mäusen, die mit suboptimalen Antigendosen geimpft wurden, erhöhte Polymannuron-Guluronsäure (Alginsäure) die Anzahl der 19S-antikörperbildenden Zellen in der Milz bis auf das 44-fache.

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[5] A. G. JOHNSON, J. SCHMIDTKE, K. MERRITT and I. HAN, in *Nucleic Acids in Immunology* (Eds. O. J. PLESCIA and W. BRAUN; Springer-Verlag Inc., New York 1968), p. 379.

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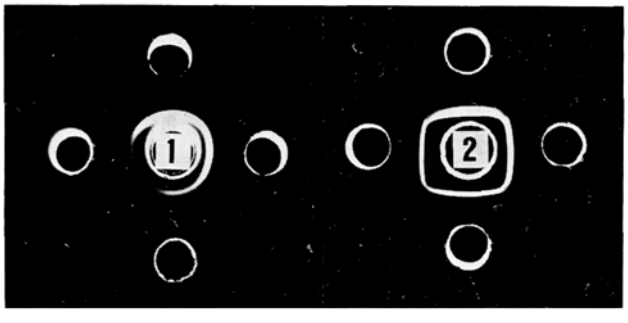
Einfluss der Ionenstärke auf das Immunpräzipitationsvermögen von Amphibien-Antikörpern

Antigen-Antikörper-Komplexe lassen sich methodisch einfach und dennoch spezifisch als Präzipitate durch das Verfahren der Immundiffusion nachweisen[1]. Von serologischen Arbeiten mit antikörperhaltigen Warmblüterseren ist jedoch bekannt, dass der Ablauf von Antigen-Antikörper-Reaktionen in vitro von der Ionenstärke des Reaktionsmilieus beeinflusst werden kann[2-4]. Das zunehmende allgemeine Interesse für die Immunitätsentwicklung bei Kaltblütern erfordert daher die Kenntnis einer eventuellen Abhängigkeit des Immundiffusionsergebnisses von der Ionenstärke[5] des verwendeten Puffermilieus, wenn statt Warmblüter-Antikörper Amphibien-Antikörper verwendet werden.

Der Vergleich von Kaninchen-Antikörpern und Erdkröten-Antikörpern in ihrem Immunpräzipitationsverhalten mit Rinderalbumin als Antigen in Ouchterlony-Platten (0,85% Ion-Agar in Sörensen-Phosphatpuffer mit Ionenstärke I = 0,150; 0,100; 0,050; 0,025; 0,005 oder in Aqua bidestillata, d.i. I = 0,000) ergab unterschiedliche Abhängigkeiten von der jeweils vorliegenden Ionenstärke. Während nämlich das Auftreten von Rinderalbumin-Kaninchen-Antikörperpräzipitaten ohne wesentliche Unterschiede über den gesamten I-Bereich von 0,000–0,150 verteilt war, traten optimale Präzipitate mit den Kröten-Antikörpern nur in zwei verschiedenen Berei-



Präzipitationslinien nach einer Reaktion von Kröten-Antikörpern (1) bzw. Kaninchen-Antikörpern (2) mit Rinderalbumin verschiedener Konzentrationen.

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