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On the Role of T Lymphocytes in Stimulation of Humoral Immunity Induced by Peptidoglycan-Monomer Linked with Zinc

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Key Words
Peptidoglycan monomer linked with zinc · Sheep red blood cells · Plaque-forming cell · IgM · IgG · IgG1 · IgG2a · T subset depletion, in vivo · Cellularity, bone marrow, thymus, spleen · Lymphocytes, CD4+, CD8+, CD5+ · FACS analysis · Thymus-independent antigen-2

Abstract
Effects of peptidoglycan linked with zinc (PGM-Zn) were investigated on plaque-forming cell (PFC) generation to sheep red blood cell (SRBC) and SRBC-unrelated antibody production in primary and secondary immune response in mice depleted in vivo of CD4+ and/or CD8+ T lymphocytes. PGM-Zn in nondepleted mice stimulated the PFC generation and IgM or IgG and IgG1 production in primary and secondary reaction. Single depletion of CD4 or CD8+ T cells did not change this ability. The effects of PGM-Zn after CD8+ depletion were even greater than those in nondepleted mice. Depletion of both T cell subsets, however, completely abrogated immunostimulatory effects of PGM on PFC generation (primary and secondary response), as well as on primary SRBC-unrelated antibody production, leaving only the increase of IgG in secondary response unchanged. Immunostimulatory effects and isotype switching to IgG1 and IgG2a correlated with the changes in splenic CD4+, CD8+, CD5+ cells, pointing to the regulatory role of these cells and/or their cytokines in PGM-Zn-induced immunostimulation. Altogether the data suggest that PGM-Zn may potentiate the costimulatory signals coming from activated T cells and act on B cells without the T cell help.

Introduction

Bacterial products such as lipopolysaccharide (LPS) or peptidoglycan (PG) isolated from gram-negative or gram-positive bacteria, respectively, are well known immunomodulating agents capable of influencing the immune reaction generated upon antigen or pathogen stimulation. As substances which are constantly produced in low levels by microbial flora, they are important for functioning of the entire immune system, but in higher doses they become the dangerous initiators of multiorgan failure and lethal shock [1]. Intensive research on the mechanisms of their action is therefore directed both to find immunoadjuvants with better immunostimulating activity and to find substances which may prevent their high toxicity in septic conditions.

Chemically, PG constitutes a polymer of alternating β1, 4-linked *D*-GlcN and N-acetylmuramic acid residues, forming the three-dimensional network by interlinked peptide bridges. Muramyl dipeptide was identified as the minimal structural unit of PG expressing bioactivity, but the peptidoglycan monomer (PGM) which has similar activities, was originally prepared by biosynthesis from culture fluids of penicillin-treated *Brevibacterium divaricatum* NRRL-2311

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[2]. Its immunomodulating properties were repeatedly emphasized. PGM enhances humoral immunity, retards growth and spread of mouse melanoma, Lewis lung and mammary carcinoma, and methylcholanthrene-induced fibrosarcoma, stimulating macrophages, T and B cells, and NK cells in mice [3–6]. Even better immunostimulating activities were obtained by PGM-Zn, an analogue of PGM in which the basic substance (PGM) was linked with zinc [7, 8], which by itself also possesses immunostimulating properties [9, 10]. We have shown, previously, that prolonged treatment of old mice with this metal complex of PGM may correct the age-associated decline of immune functions and induce the appearance of peritoneal macrophages with suppressive activities on spontaneous and ConA-induced blastic proliferation in vitro [7, 8]. Immunostimulating activities of PGM-Zn were also obtained in mice which were immunosuppressed by obstructive jaundice [11], and by halothane anesthesia [12]. Owing to these data, suggesting that PGM-Zn has useful immunoadjuvant activities, in the present study an attempt was made to obtain more information about the mechanism of its action. For this purpose we analyzed the effects of PGM-Zn in mice which were in vivo depleted of CD4+ and/or CD8+ T cells, and thus subjected to a selective type of immunodeficiency.

The results showed that immunoregulatory effects of PGM on splenic PFC generation to sheep red blood cells (SRBC) and serum antibody production might be both T-cell-dependent (TD) and T-cell-independent (TI), indicating its ability to enhance the generation of costimulatory signals and induce the polyclonal B cell activation.

Material and Methods

Mice

In all experiments we used inbred, 2- to 3-month-old, male mice oft the BALB/c strain. They were originally obtained from Jackson Laboratory and bred in the Central Animal Facility at the Medical Faculty in Rijeka, Croatia. During the experiments, the animals were kept in standard plastic cages, fed with standard mouse pellets and water ad libitum and exposed to the natural day-light cycle.

In vivo Depletion of T Cell Subset

Depletion of both T lymphocytes subsets was accomplished by in vivo treatment of mice with rat antimurine CD4 (YTS 191.1; IgG2b) and rat antimurine CD8 (ITS 169.4; IgG2b) monoclonal antibodies (mAbs), respectively. Antibodies were purified from hybridoma-growing ascitic fluid, and the percent of specific depletions estimated at the level of spleen was 86 and 90% [13]. Mice were injected with mAbs intraperitoneally (1 mg of immunoglobulin per animal, diluted in 0.5 ml of PBS i.p.) 2 days before immunization with SRBC and treatment with PGM-Zn. Mice in the control group were injected with rat serum.

Treatment with PGM-Zn

The immunomodulating agent PGM [GlcNAc-MurNAc-*L*-Ala-*D*-iso-Gln-meso-diamminopimelic acid (w-NH_2)-*D*-Ala-D-Ala] linked with zinc (PGM-Zn, 'Pliva'-Zagreb) was prepared by biosynthesis from the culture fluids of *B. divaricatum* NRRL-2311, as an apyrogenic, water-soluble substance, devoid of any toxic effects [2]. The sample used in this study contained less than 0.015 ng endotoxin/mg PGM, according to the limulus amebocyte lysate test (Pyrostat Kit, Millipore). Mice were injected with PGM-Zn dissolved in PBS (10 mg/kg of body weight, i.p.), immediately after the sensitization with SRBC, which was done 2 days after the depletion of T cell subsets. Mice were sacrificed 4 days later, or resensitized on the 10th day with SRBC for the evaluation of primary or secondary immune response, respectively. At time of resensitization a second injection of the same dose of PGM-Zn was given intraperitoneally. Mice in the control groups received the same volume (0.5 ml) of PBS.

Cell Separation

Murine spleen and thymuses were aseptically removed from experimental animals and gently pressed through a fine stainless steel screens in RPMI 1640. Red blood cells were lysed with Tris-buffered ammonium chloride for 5 min. After washing, the cell suspension was filtered through a fine nylon mesh, resuspended in complete medium and adjusted to the desired final concentration for FACS analysis or for the plaque-forming cell (PFC) response. Suspensions of bone marrow cells were prepared by injecting saline into the medullar channels of femur diaphysis after eliminating the epiphyses.

PFC Response

Mice were immunized by intraperitoneal injection of SRBC (10% in saline) and 4 days later the primary immune response was evaluated by determination of hemolytic PFC production by the method of Cunningham and Szenberg [14]. Secondary immune response was tested on day 14 in mice resensitized with SRBC 10 days after the first sensitization.

ELISA for Detection of Immunoglobulin in Serum

Antibody titers of Ig in sera of experimental animals were determined by sandwich ELISA [15]. For this purpose, 96-well microtiter plates were coated with rabbit antimouse antibodies (10 mg/ml; Jackson Immuno Research Lab.), diluted in carbonate buffer (pH 9.2). Plates were incubated for 24 h at +4 °C, and then washed twice with a mixture of PBS, Tween 20 (0.05%; Sigma) and FCS (1%). Residual binding capacity of the plate was blocked with gelatine diluted in distilled water (1:10). After incubation for 1 h at 4 °C and double washing with PBS and Tween 20, test serum was added in the initial concentration, and then serially diluted. After serial incubation (45 min), addition of secondary rat antimouse antibodies conjugated with peroxidase, and repeated washing of unbound antibodies, substrate OPD (*o*-phenylene-diamine; Sigma) and H_2O_2 were added. The reaction was stopped by 1 *N* H_2SO_4, and optical densities of samples were read using an automatic microtiter plate reader (ELISA processor II, Behring). Concentrations of antibodies in tested sera were quantitated by extrapolation onto the curve generated with serial dilutions of the standard antibody solution.

Cytofluorometric Analysis

The surface phenotype of cells in spleen was identified by direct immunofluorescence analysis on FACScan (Becton Dickinson, Immunocytometry Systems, Mountain View, Calif.), using FACScan

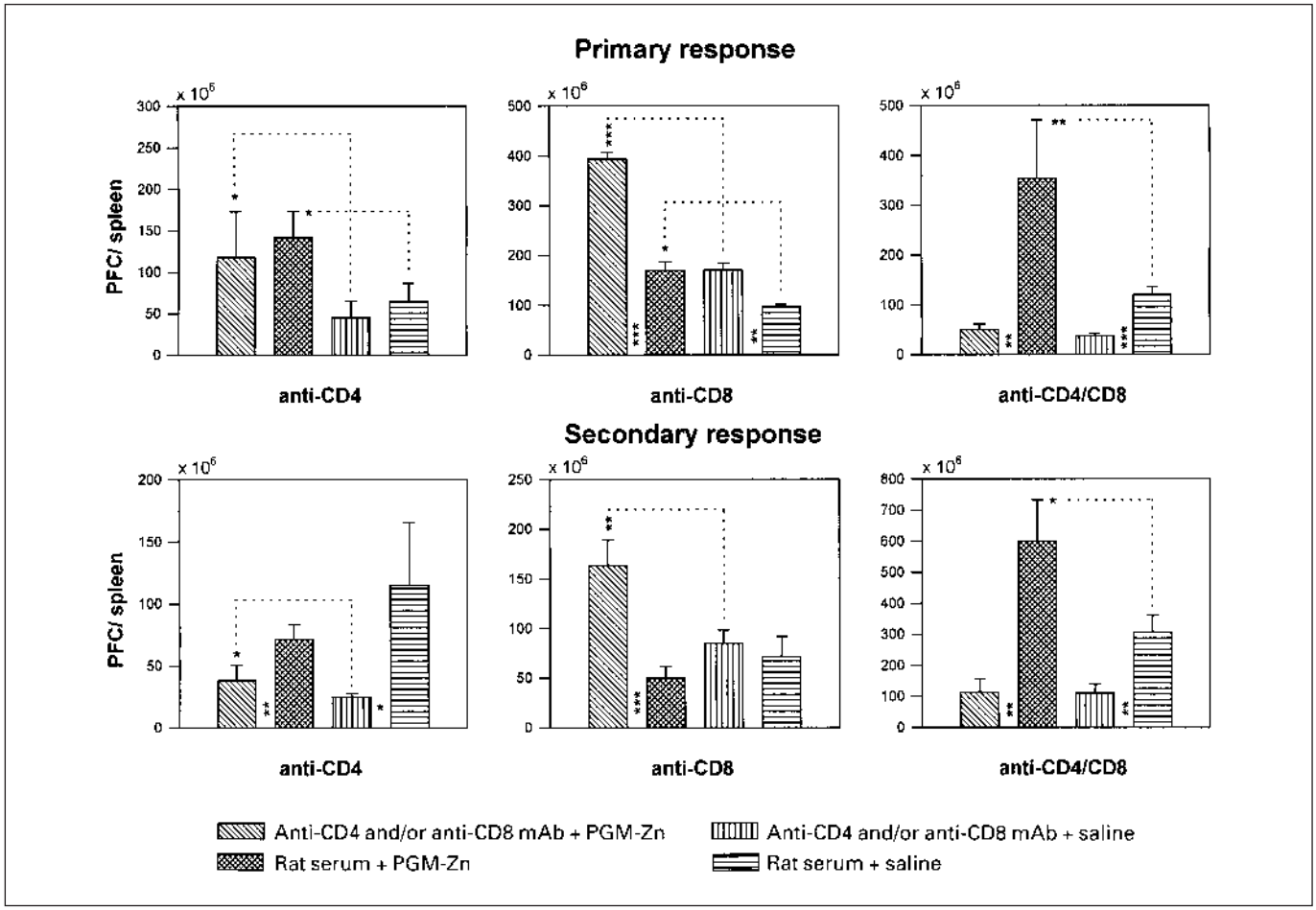

Fig. 1. Effects of PGM-Zn on primary and secondary PFC production in CD4 and/or CD8-depleted BALB/c mice sensitized with SRBC (as described in Material and Methods). Mice in the control groups were subjected to the same injection procedure using rat serum (instead of mAbs) or saline solution (instead of PGM-Zn). Each column represents mean ± SE (n = 6). *$p<0.05$; **$p<0.01$; ***$p<0.001$.

Research Software. For this purpose the suspension of spleen cells was prepared by pressing the tissue fragments through a nylon mesh, then washed twice in RPMI 1640, and exposed to ammonium chloride to lyse the erythrocytes. After rewashing, the cell number was adjusted to 1×10^6/ml. This suspension was centrifuged and the cells were resuspended in 50 ml of cold FACES medium and were incubated with 1 mg of primary mAbs dissolved in the same volume (PE-conjugated goat antimouse CD4 and CD8, or FITC-conjugated goat antimouse CD5 and immunoglobulin (Ig), Becton Dickinson, Mountain View, Calif.). After 30 min of incubation at +40 °C, and removing the unbound antibodies by washing in FACS medium, the cells were finally resuspended in 1 ml of FACS medium. Propidium iodide (1 mg/ml)-stained dead cells were excluded by electronic gating.

Statistical Analysis

The data were analyzed using the Sigma Plot Scientific Graphing System, Version 4. 03. Statistical significance was calculated by two-tailed Student's t test for unpaired samples. $p<0.05$ was considered significant. Data are reported as mean ± SEM, unless otherwise noted.

Results

Effects of T Cell Depletion and PGM-Zn Administration on Antigen-Specific Immune Response

Primary and secondary responses to the TD antigen (Ag)-SRBC were evaluated in PGM-Zn-treated mice, depleted of CD4+, CD8+, and of both CD4+ and CD8+ T lymphocytes, or in control mice, similarly treated with saline (as a control for PGM-Zn injection) or with rat serum (as a control for treatment with mAbs). Elimination of CD4+ T cells did not affect the splenic PFC generation in primary response, but inhibited their production after resensitization with SRBC (fig. 1; columns 3 vs. 4). Elimination of CD8+ T cells stimulated primary PFC production, while depletion of both CD4+ and CD8+ T cells completely abrogated the response to SRBC, confirming the regulatory role

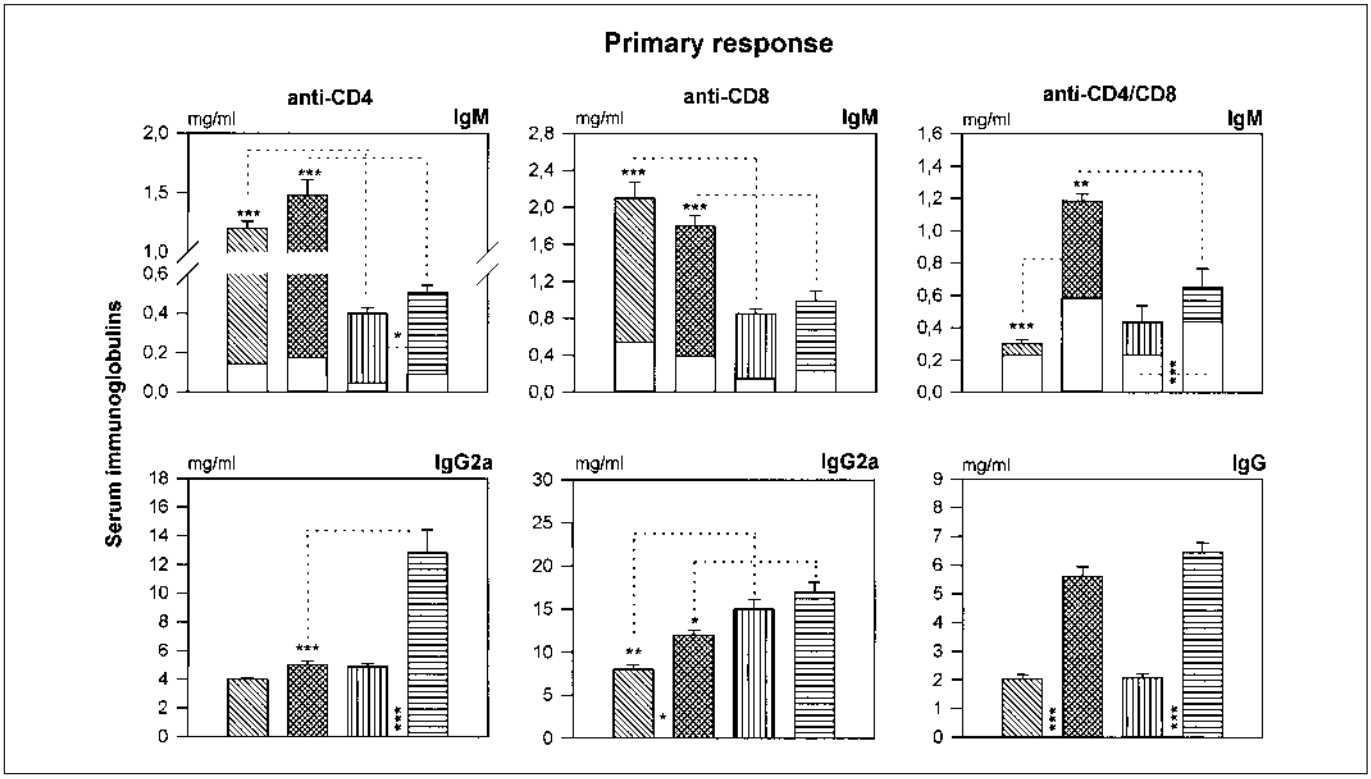

Fig. 2. Effects of PGM-Zn on the concentration of SRBC-specific (empty columns) and unspecific (shaded columns) antibodies in serum during the primary immune reaction in mice sensitized with SRBC. Results are expressed as mean ± SE (n = 6). Shading is explained on figure 1. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

of T subsets in the reaction to TD Ag. PGM-Zn in nondepleted mice repeatedly stimulated PFC generation in primary response and produced variable effects on secondary response (fig. 1; columns 2 vs. 4). Single depletion of CD4 or CD8+ T cells did not change the ability of PGM-Zn to stimulate both types of immune response, and its effects after CD8+ depletion were even greater ($p < 0.001$) than those in nondepleted mice (fig. 1; columns 1 vs. 3). However, in mice depleted of both T cell subpopulations PGM-Zn was ineffective, and unable to correct the inhibitory effects of depletion in both the primary and secondary type of immune response to SRBC. Measurement of the concentration of Ag-specific IgM immunoglobulin in the serum of CD4 and/or CD8-depleted mice during the primary immune response to SRBC gave similar results (fig. 2), as those obtained by analysis of PFC in the spleen, confirming the regulatory role of T cells in these events.

Effects of T Cell Depletion and PGM-Zn Administration on Serum Ig and Isotype Regulation

To obtain an insight into the effects of PGM-Zn on Ag-unspecific antibody production, and on Ig isotype regulation, in the serum we also detected the total Ig isotype level during the primary or secondary response, presented as titers of IgM, IgG, IgG2a or IgG1. The data revealed that PGM-Zn during the primary immune response in nondepleted and single CD4 or CD8-depleted mice enhanced not only the titer of Ag-specific IgM antibodies, but also the Ag-unspecific synthesis (fig. 2; columns 2 vs. 4 and 1 vs. 3, respectively). Depletion of both types of T cells, however, similarly as shown by PFC analysis on figure 1, markedly lowered IgM (Ag-specific) and total IgG production (fig. 2; columns 3 vs. 4), and completely abrogated the immunostimulating activity of PGM-Zn (fig. 2; columns 1 vs. 2). Simultaneously, the isotype analysis showed that enhancing effects of PGM-Zn on IgM production in nondepleted and single depleted mice was followed by a decrease in the concentration of IgG2a (fig. 2; columns 2 vs. 4). In the same groups, the similar, T-cell-dependent immunostimulatory

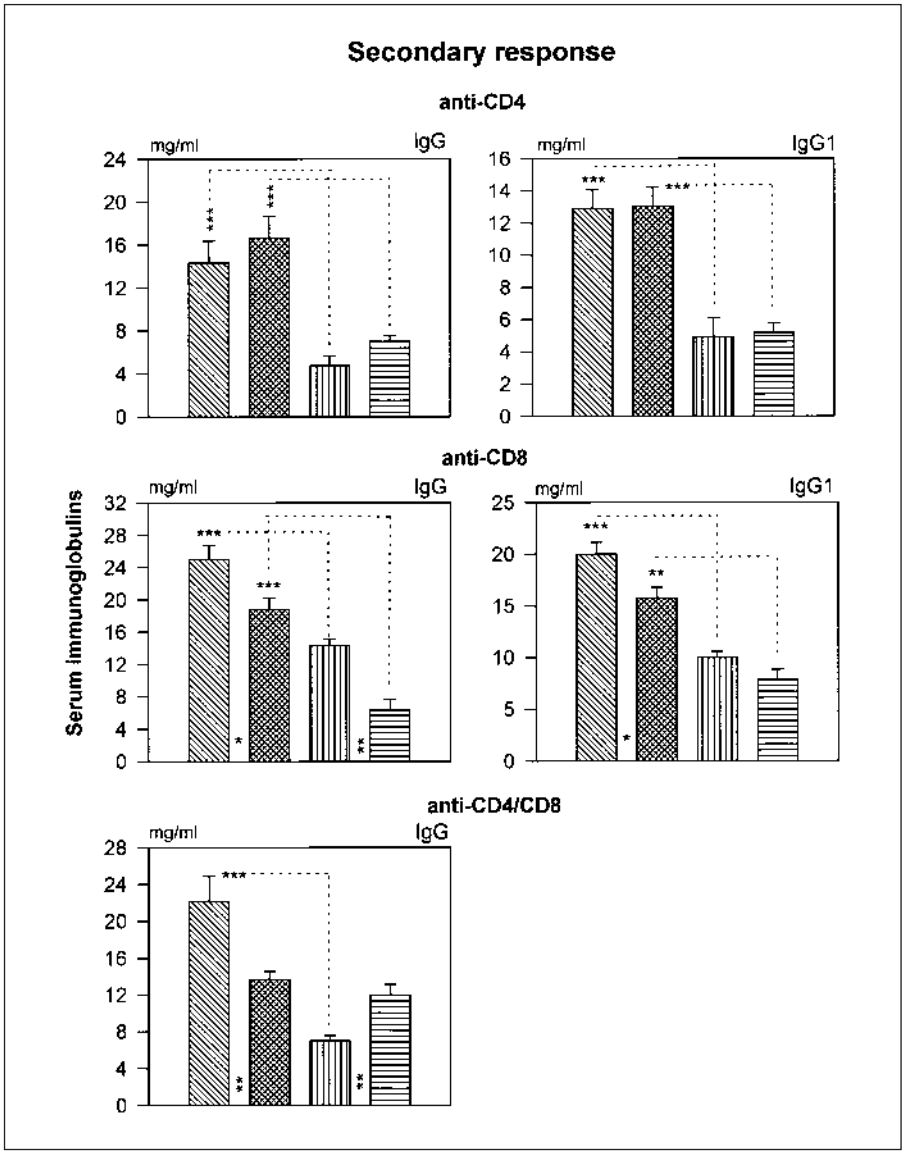

Fig. 3. Effects of PGM-Zn on the concentration of antibodies in serum during the secondary immune reaction in mice sensitized with SRBC. Results are expressed as mean ± SE (n = 6). Shading is explained on figure 1. *p < 0.05; **p < 0.01; ***p < 0.001.

effects of PGM-Zn was also observed in the secondary type of immune response on the total levels of IgG and IgG1 (fig. 3; columns 1 vs. 3 and columns 2 vs. 4). Depletion of CD8+ T cells similarly, as was noticed for secondary type of PFC generation (fig. 1) even potentiated this stimulation (fig. 3; columns 1 vs. 2). However, in contrast to the previously described evidence, which showed that enhancing effects of PGM-Zn on serum immunoglobulins in primary immune reaction and on secondary PFC generation might be abrogated by elimination of both T cell populations (fig. 2, 1, respectively; columns 1 vs. 3), in the secondary type of immune reaction, after double depletion, an increase of IgG levels (fig. 3; columns 1 vs. 3) was noticed, suggesting that in this aspect the effects of PGM-Zn might be T-cell-independent.

Cellularity of Lymphatic Organs and Phenotypic Profile of Lymphatic Cells after the Treatment with PGM-Zn

Simultaneously with the experiments described, the cellular profile of lymphoid organs was analyzed. In mice depleted of the CD4+ or CD8+ T cell subpopulation PGM-Zn significantly increased the cellularity of bone marrow, thymus and spleen (fig. 4). The hyperplastic effects of PGM-Zn on spleen and bone marrow were, however, completely absent in double-depleted mice. Phenotypic analysis in spleen (fig. 5) showed that after the depletion of one of the

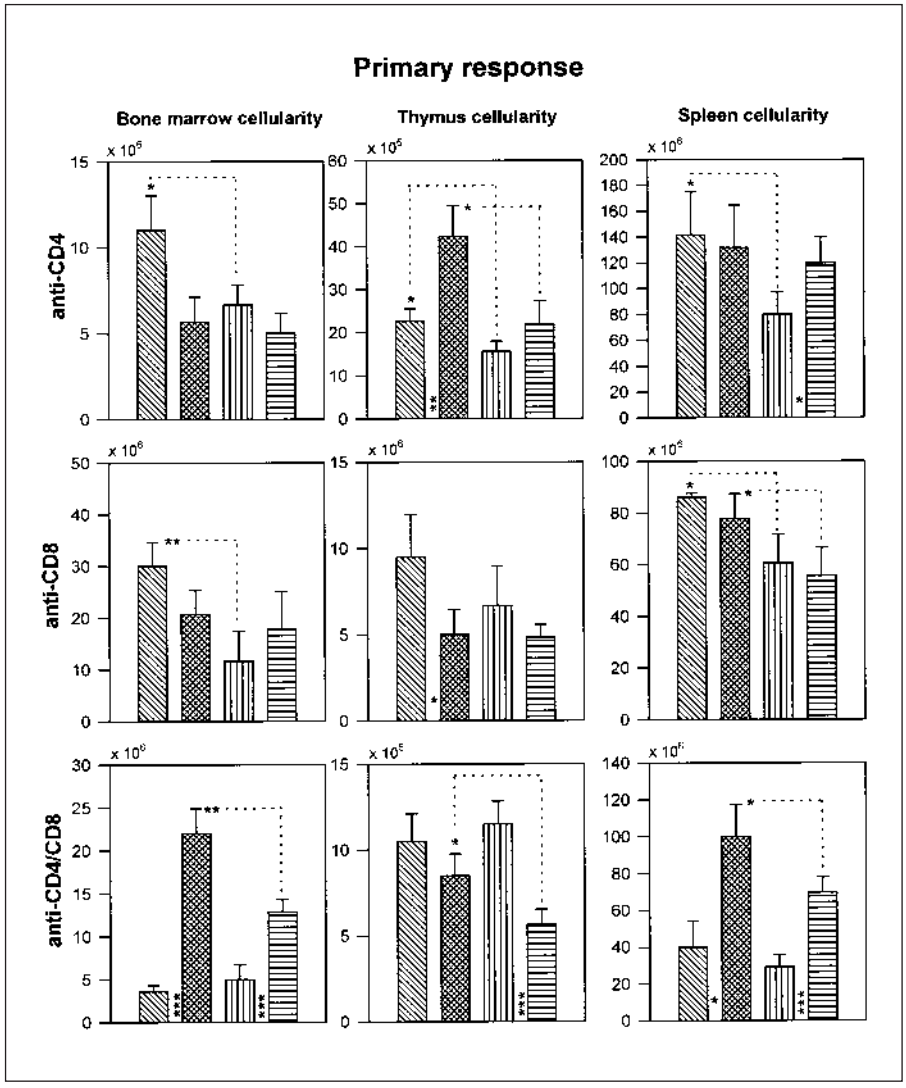

Fig. 4. Effects of PGM-Zn on the cellularity of lymphatic organs in CD4 and/or CD8-depleted mice 4 days after the treatment with PGM-Zn and sensitization with SRBC. Results are expressed as mean ± SE (n = 6). Shading is explained an figure 1. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

T cell subpopulations, PGM-Zn, markedly increased the number of the remaining CD4+ or CD8+ cells, respectively, as well as the CD5+ cells. Furthermore, the absence of splenic CD4+, CD8+, CD5+ cells found during the primary immune response in double-depleted mice correlated with the absence of an enhancing effect of PGM-Zn on PFC generation (fig. 1) and on serum antibody production in the primary type of immune response (fig. 2), indicating the role of cells with these phenotypes in the PGM-Zn-induced immunostimulation. In a secondary type of immune response in all groups the phenotypic profile of cells in spleen was similarly changed as during the primary response, but in double depleted mice PGM-Zn significantly increased the cellularity of bone marrow (data not shown).

Discussion

In contrast to the relatively well known mechanisms of action of endotoxin (LPS), which is released from the bacterial cell wall of gram-negative bacteria, the mechanisms through which the target cells are activated by gram-positive microbial components (such as peptidoglycan, lipoteichoic and teichoic acid) are less well understood. The mechanisms of action of PGM and its new metal complex PGM-Zn, which was synthesized in an attempt to combine the immunostimulating properties of each of its constituents, are even less known. The biologically active PGM possesses the linear non-cross-linked structure made of repeating disaccharide-pentapeptide units [2]. When parenterally administered to the mice PGM is hydrolyzed by the en-

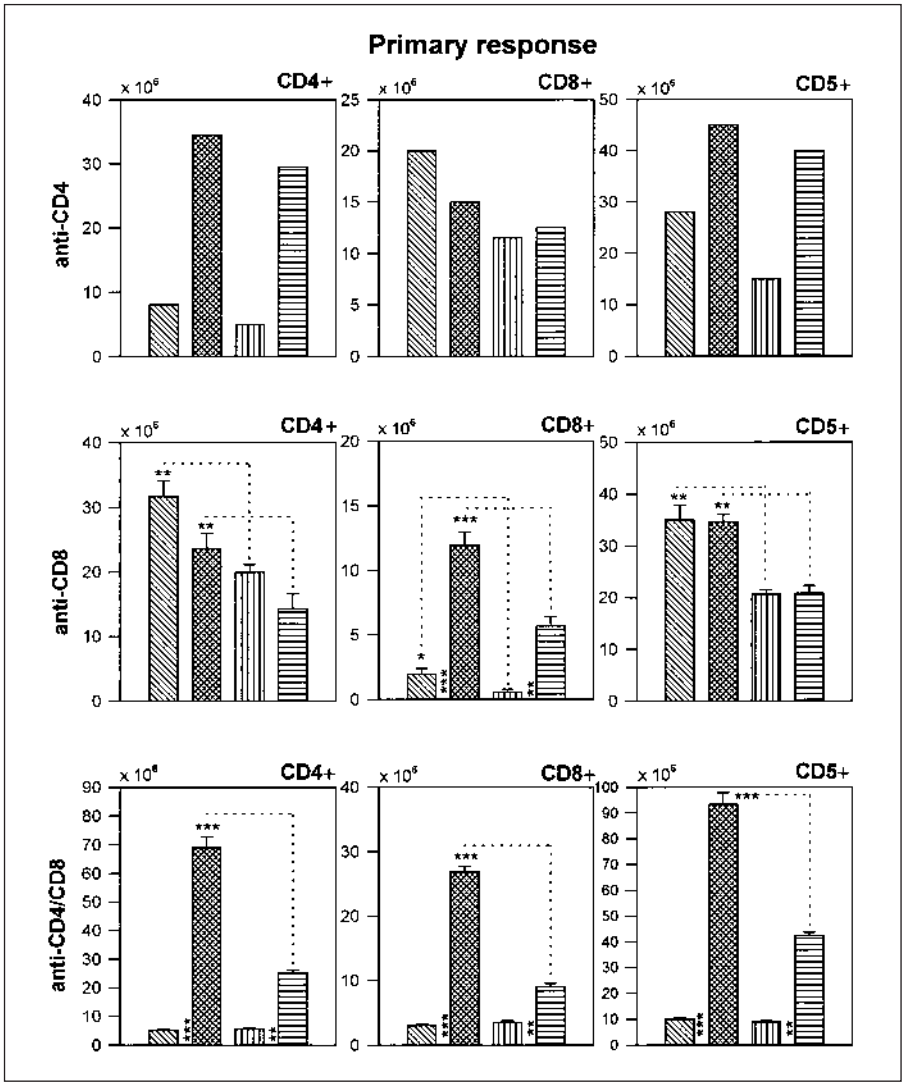

Fig. 5. Phenotypic profiles of spleen cells (presented as total number) 4 days after the treatment with PGM-Zn and sensitization with SRBC. Results are expressed as mean ± SE (n = 6). Shading is explained on figure 1. *p < 0.05; **p < 0.01; ***p < 0.001.

zyme N-acetylmuramyl-*L*-alanine amidase to the disaccharide and pentapeptide moiety [16]. The metabolic features of PGM-Zn are not known, but our previously reported data have shown that its immunoregulating activities might also be linked with Zn^{2+}, since the effects of PGM-Zn were not identical to those of PGM [7]. In adult mice it had greater effects on hepatic class II expression and induced the PFC generation to SRBC, whose intensity negatively correlated with the total quantity of hepatic protein [7]. Additionally, we noticed that PGM-Zn has a greater capacity than PGM to induce the generation of peritoneal mononuclear cells with suppressive activities [7, 8].

The results from this study indicate that PGM-Zn in mice sensitized with SRBC may stimulate both the Ag-dependent and SRBC-unrelated type of antibody production, by mechanisms which might be both dependent on CD4+ and CD8+ T cells, and independent of T cells. In nondepleted mice it increased primary and secondary PFC production (fig. 1) and markedly enhanced serum levels of Ag-specific and unspecific IgM in primary (fig. 2) and IgG and IgG1 in secondary immune reaction (fig. 3). These enhancing effects of the PGM-Zn persisted in single CD4+ or CD8+-depleted mice. However, in mice depleted of both T subpopulations its effects on PFC generation and serum IgM and IgG in primary immune response were completely lost, pointing to the requirement of the T cell help and cytokine costimulation for the full effects of PGM-Zn. In the absence of CD4 and CD8+ subsets, the T cell-independent reaction was only the enhancing effect of PGM-Zn on IgG production in secondary immune reaction (fig. 3), probably sug-

gesting the greater effect of PGM-Zn on mature B lymphocytes. Taken together, it is, therefore, likely that PGM-Zn acts as adjuvant, which enhances the costimulatory signals in T cells, but which also behaves as a TI Ag, able to induce polyclonal B cell activation.

The presented data do not make it possible to define the type of TI Ag to which PGM-Zn might belong. However, owing to its effect on isotype switching (fig. 2, 3) and persistence of its effects in double depleted mice in a secondary type of immune reaction (fig. 3), it could be speculated that it has some characteristics of TI-2 Ag. As it is well known TI-1 Ag contain an intrinsic activity that directly induces the proliferation of B cells, but are inefficient inducers of isotype switching, while, the activity of TI-2 Ag, which activate only mature B cells, might be greatly enhanced by T_H2 cytokines [17]. Response to several TI-2 Ag has been shown to be dominated by CD5 B cells that normally comprise only a minor subpopulation of B cells [18]. However, although in our study the immunostimulating effects of PGM-Zn on the IgM level in primary response highly correlated with the presence of CD5+ cells in the spleen (fig. 5), in the secondary immune response we found an increased titer of IgG (fig. 3; double-depleted mice) also during the absence of these cells, and splenic CD5+ cells were eliminated by the use of anti-CD4 and anti-CD8 mAbs, indicating their T lineage origin. There is a certain possibility that in the hyperplastic bone marrow of PGM-Zn-treated, double depleted mice self-renewing CD5+ B cells were also present, but this remains to be clarified.

As it is known, B cell activation by TI-2 Ag requires or is greatly enhanced by cytokines, produced mostly by helper (T_H2) T cells which also lead to isotype switching [17]. It is likely that some of our data obtained in mice depleted only of CD4+ or CD8+ T lymphocytes support the above-mentioned possibility. Namely, in CD8-depleted mice, an enhanced effect of PGM-Zn on PFC generation and serum antibodies production was seen (fig. 1, 2), combined with the low concentration of IgG2a (fig. 3) and increased levels of IgG1 (fig. 4). These effects in SRBC-sensitized mice, obviously, could be explained by the elimination of CD8+ T cells with suppressive activities and prevalence of helper T (T_H2) lymphocytes and their cytokines among the remaining CD4+ cells. As known, CD4+ subset may be subdivided into two categories based in their functional pattern of secreted cytokines [19, 20]. They differentially affect the process of isotype switching and simultaneously inhibit the action of the opposite cell subtype [21, 22]. Since T_H2 cytokines preferentially induce the switching toward the IgG1 isotype and inhibit the production of IgG2a antibodies, while the predominance of T_H1 cytokines induces switching to IgG2a and inhibits the IgG1 expression [22], it could be that a low concentration of IgG2a (fig. 3) and increased levels of IgG1 found in our CD8-depleted mice (fig. 4) were the consequence of both a higher concentration of IL-4 released from activated T_H2 lymphocytes, or a lower concentration of IFN-γ, which was less released from inhibited T_H1 cells.

Furthermore, it seems that some data obtained in CD4-depleted mice also point to the involvement of T_H2 cells in PGM-Zn induced immunostimulation. Namely, in these mice regardless of CD4 depletion PGM-Zn was able to stimulate PFC generation (fig. 1) and IgM production in primary (fig. 2) as well as IgG (fig. 3) and IgG1 antibody production (not shown) in secondary response suggesting that after the use of anti-CD4 mAbs were not eliminated T_H2, but primarily T_H1 lymphocytes. In proof of this in CD4-depleted, PGM-Zn-untreated mice, a very low level of IgG2 concentration (T_H2-dependent effect) in primary immune reaction was also found (fig. 3). This assumption is in agreement with the finding that in vivo treatment of mice with anti-CD4 mAb spare the activated CD4+ cells, and even result in the B cell polyclonal activation owing to the enhancement of T_H2-like functions [23, 24].

Our data also point to the stimulatory effects of PGM-Zn on bone marrow, thymus and spleen (fig. 4). In CD4- or CD8-depleted mice it induced compensatory hyperplasia of the remaining T cell subpopulation with CD8 or CD4+ phenotype, respectively (fig. 5). It could be hypothesized that among the remnant cells after the use of anti-CD4 or anti-CD8 mAbs were also some gamma/delta or CD4–CD8– T cells which recognize a component of the polysaccharide antigen bound to unconventional MHC class I or class I-like molecules [25, 26], or probably NK cells, which may be activated directly by polysaccharides through their own specialized receptors [27]. The role of NK cells in PGM-Zn-induced stimulation of humoral immunity remains to be clarified, but there is a possibility that in our double-depleted mice, not only CD4+ and CD8+ T cells, were eliminated, but also some CD4+NK1.1+, which in mice have a specific capacity to deliver B cell help, as an early source of IL-4 necessary for later T_H2 differentiation and their IL-4 production [28]. In support of this mice which lack both T and NK cells have a serum level of polysaccharide-specific IgG3 reduced by 90% [29], and markedly reduced mortality in the LPS-induced Shwartzman reaction [30].

In the same experimental model we observed that the ability of PGM-Zn to induce upregulation of the MHC class II antigen expression in the liver is also TD [unpubl. data]. These, and previously published data [7, 8], therefore, suggest that PGM-Zn might also activate the macrophages,

which through production of cytokines such as IFN-γ and GM-CSF [27, 29], or prostaglandins [31] and nitric oxide [32] may further influence the intensity of immune response and the process of isotype switching.

Most of these cells potentially activated by PGM-Zn express the specific binding sites for bacterial cell wall constituents [1, 33]. However, for now it is unclear to which of the PGM-Zn components the observed effects might be linked, since released PGM-Zn fragments include disaccharide-pentapeptide moieties and Zn ions, which might all have immunomodulatory properties [9, 10, 34, 35]. To resolve some of the crucial questions about the mechanisms of its action, in further experiments we plan to investigate the effects of PGM on monocytes and its relationship to membranous and soluble CD14, as well as the role of T cells in these events. Preliminary in vitro obtained data suggest that cellular receptors for PGM and LPS on mononuclear lymphatic cells may be the same, while in vivo obtained data showed that its enhancing effects on hepatic MHC II expression and phagocytic activity of peritoneal macrophages might also be dependent on CD4+ and CD8+ T cells (unpubl. data). Therefore, it is possible that this new adjuvant may act not only on B cells, but also enhance the uptake of the antigen by Kupffer cells or act by stimulating the expression of costimulatory activity in Ag-presenting cells, as well as by mimicking the costimulatory signals in T cells.

In conclusion, despite the unclear mechanisms of the action, our data show that PGM-Zn in mice sensitized with SRBC has adjuvant effects on splenic PFC generation and on SRBC-unrelated antibody production in serum in primary and secondary immune response. The Ag-specific reaction and antibody production in primary response were maintained after the depletion of CD4 or CD8+ T lymphocytes, but were completely abrogated after the depletion of both T cell subsets, suggesting that PGM-Zn potentiates the costimulatory signals coming from activated T cells. PGM-Zn-induced antibody production in secondary immune reaction was, however, T cell-independent, pointing to its greater effects on mature B cells and suggesting that PGM-Zn might also have some characteristics of TI-2 Ag. Taken together, the data also emphasize that the combination of PGM and Zn gave a new immune stimulatory complex with adjuvant activity which might also be useful in some clinical states of T cell deficiencies.

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