G_2-chalone was 20, 25, and 35 kilodaltons [4, 9, 10]. It should be pointed out that molecular weight was determined in the studies cited above by gel-filtration and ultracentrifugation; moreover, only partially purified chalones obtained from different species of animals were studied.

By immunoaffinity chromatography using antibodies of a monospecific immune serume it was thus possible to isolate epidermal G_2-chalone from an alcoholic extract of skin in one stage. It can evidently be obtained by this method also from an aqueous extract of skin without preliminary fractionation with alcohol, and the whole procedure of purification can be reduced to a minimum. The method of purification used, which has been successfully applied for obtaining various substances, proved also to be suitable for epidermal G_2-chalone. The purified inhibitor was biologically active and preserved one of the main properties of chalones, namely tissue specificity of action.

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STIMULATION OF DNA SYNTHESIS IN SPLENIC LYMPHOCYTES OF GUINEA PIGS BY STAPHLYOCOCCAL PEPTIDOGLYCAN

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The study of the role of staphylococcal antigens in immunologic reactions is of both theoretical and practical importance, for it can deepen our knowledge of interaction between individual structures of bacteria and lymphocytes of the host. Several investigators have shown that the peptidoglycan in the cell wall of Gram-positive and Gram-negative bacteria has mitogenic activity against lymphocytes of man, rats, and mice [7-10]. Formation of the immune response and the ability of the lymphocytes to respond to mitogens are linked with the degree of differentiation of the lymphocytes and the organization of their receptor apparatus [1, 6, 11].

With these considerations in mind it was decided to study DNA synthesis in splenic lymphocytes of intact guinea pigs in response to peptidoglycan and to compare it with that observed in the presence of nonspecific B and T cell mitogens, and to identify the lymphocyte subpopulation on which peptidoglycan acts.

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TABLE 1. DNA Synthesis in Splenic T and B Lymphocytes of Guinea Pigs in Response to Mitogens and Peptidoglycans J-i (M ± m, P = 95%)

Mitogen	Dose of mitogen in 1 ml culture medium	Incorporation of [^{3}H]thymidine into DNA of spleen cells treated with reagents, cpm					
		culture medium	complement	anti-IgG-serum	ATG	Anti-IgG-serum + complement	ATG + complement
Control	—	2 394±345 (1 360—3 428)	2 714±271 (2 059—3 370)	1 456±199 (955—1 957)	1 895±156 (1 483—2 307)	2 907±238 (2 275—3 539)	1 669±147 (1 200—2 138)
LPS	100 μg	28 518±2 791 (20 145—36 891)	22 542±1 791 (18 208—26 876)	9 740±763 (8 356—11 124)	12 917±300 (10 000—14 568)	5 917±312 (5 080—6 754)	11 126±988 (8 025—14 227)
PHA	0.1 μg	38 180±2 449 (30 743—45 617)	30 908±5 331 (15 618—46 198)	34 396±1 619 (30 478—38 314)	19 605±878 (17 743—21 467)	42 123±1 474 (38 633—45 613)	10 681±681 (8 957—12 405
Con A	25 μg	65 856±3 294 (55 974—75 738)	57 903±5 204 (47 498—68 308)	51 612±1 827 (47 191—56 033)	31 722±2 507 (34 387—39 057)	52 402±8 173 (34 404—70 400)	21 013±2 404 (15 378—26 648)
Peptidoglycan	100μg	34 152±2 626 (26 274—42 030)	27 564±1 760 (23 305—31 823)	16 516±988 (14 125—18 907)	32 495±951 (29 659—35 331)	18 189±1 268 (15 062—21 316)	29 486±785 (27 251—31 721)

Legend. Anti-IgG-serum denotes rabbit anti-IgG-serum against guinea pig IgG; ATG denotes rabbit antithymocytic globulin against guinea pig thymocytes. Confidence interval shown in parentheses.

EXPERIMENTAL METHOD

To obtain peptidoglycan museum strain *Staphylococcus aureus* 2287 was used. Peptidoglycan was extracted from the cell wall of the staphylococci with TCA [5]. Rabbit anti-IgG-serum against guinea pig IgG, generously provided by K. L. Shakhanina, rabbit antithymocytic globulin (ATG) against guinea pig thymocytes, obtained by the method described previously [2], and fresh guinea pig complement were used The effectiveness of the antiserum and ATG was verified in the cytotoxic test [6] in the presence of complement (dilution 1:5). To eliminate T cells, dilution of ATG was used: this caused lysis of 85-90% of thymoxytes and 45% of spleen cells in the presence of complement. Under these conditions ATG had no significant effect in the presence of complement on the viability of guinea pig bone marrow cells. Anti-IgG-serum, in the presence of complement, lysed 80-87% of bone marrow cells and 40-43% of spleen cells but had no effect on the viability of guinea pig thymocytes.

Experiments were carried out on spleen cells of intact guinea pigs. The spleen cells were obtained and cultured in the blast transformation reaction (BTR) by the method described in [3]. Depending on the experimental conditions, the cells were incubated in medium either with anti-IgG-serum or with ATG for 1 h, without or with the addition of complement, washed three times, their viability was determined on the basis of uptake of 0.1% trypan blue solution, and they were resuspended in the culture medium. The cell suspension was cultured for 48 h in the presence of peptidoglycan (100 μg/ml), phytohemagglutinin P (PHA, 0.1 μl/ml, from Difco, USA), concanavalin A (Con A, 25 μg/ml, from Sigma, USA), and lipopolysaccharide from *Salmonella typhimurium* (LPS, 100 μg/ml, from Sigma). Proliferative activity of the splenic lymphocytes in the BTR was estimated from incorporation of [^{3}H]thymidine into DNA of the cells, added at the rate of 0.5 μCi per well 16 h before the end of the culture period [6].

EXPERIMENTAL RESULTS

Peptidoglycan increased the DNA synthesis in spleen cells of intact guinea pigs (Table 1). Its stimulating effect on the intensity of DNA synthesis was equal to the effect of LPS (100 μg/ml) and PHA (0.1 μl/ml), but lower than that of Con A (25 μg/ml). Treatment of the spleen cells with the complement followed by its removal had no effect on DNA synthesis in the spleen cells induced by peptidoglycan and by the nonspecific mitogens used (Table 1). Preliminary incubation of the spleen cells with anti-IgG-serum caused a decrease in the response both to LPS and to PHA ($P < 0.001$ and <0.01 respectively). Blocking the IgG receptors of the lymphocytes by anti-IgG-serum also led to a decrease in the mitogenic action of peptidoglycan. Probably the splenic Ig^+-cells, especially lymphocytes carrying IgG receptors, were sensitive to peptidoglycan.

Preliminary treatment of the cells with ATG followed by its removal from the medium had no significant effect on DNA synthesis in the lymphocytes in response to peptidoglycan and somewhat reduced the mitogenic effect of LPS, PHA, and Con A (Table 1). The results show that during stimulation of lymphocytes with peptidoglycan cells with immunoglobulin determinants on their surface, i.e., mainly B cells, are induced to proliferate. The reduction in DNA syn-

thesis in the cells after treatment with ATG in response to LPS was evidently connected with a change in the sensitivity of the B cells to mitogen [4] or, perhaps, the ATG acted not only on T cells, but also on that fraction of B cells which is sensitive to LPS.

In order to determine which lymphocyte subpopulation is stimulated by peptidoglycan experiments were carried out with elimination of B cells by means of anti-IgG-serum, and elimination of T lymphocytes by ATG in the presence of complement. Anti-IgG-serum in the presence of complement was found to weaken the mitogenic action of LPS and peptidoglycan on the cells but it did not change DNA synthesis in the lympohocytes in response to nonspecific T mitogens (Table 1). Despite the fact that anti-IgG-serum in the presence of complement significantly inhibited the response to LPS and to peptidoglycan, the level of stimulation of DNA synthesis in the cells in response to these reagents remained sufficiently high. Possibly not all Ig^+-B lymphocytes were eliminated or cells not carrying IgG receptors were drawn into mitosis. The possibility cannot be ruled out that peptidoglycan stimulates a subpopulation of B cells in the earliest or the latest stage of differentiation, which lose their Ig receptors on the cytoplasmic membrane [1, 11].

Evidence supporting the view that peptidoglycan acts principally on B lymphocytes was given by the results of experiments in which splenic T cells of the guinea pigs were eliminated by means of ATG in the presence of complement (Table 1). Treatment of the cells with ATG in the presence of complement, followed by their removal from the medium, did not affect the intensity of DNA synthesis relative to peptidoglycan, whereas DNA synthesis in the residual cell population, induced by nonspecific T mitogens, was sharply inhibited.

It can thus be concluded from these results that peptidoglycan isolated from *Staphylococcus aureus* 2287 has mitogenic activity mainly on B lymphocytes which carry Ig receptors. Unlike LPS, peptidoglycan can probably stimulate DNA synthesis in less highly differentiated lymphocytes of bone marrow origin also.

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