Short Communications

Received: June 12, 1989

Accepted after revision: November 30, 1989

Int Arch Allergy Appl Immunol 1990;91:204-206

Abolished Net Output of Lymphocytes from the Spleen after Immunization with *Salmonella typhi* H

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Abstract

Cell release from the spleen was studied by comparing the content of mono- and polynuclear leucocytes in splenic afferent and efferent blood. A difference in the content of mononuclear cells was registered in normal animals, indicating a release of such cells from the spleen. Immunization with *Salmonella typhi* H antigen reduced this veno-arterial difference and abolished it totally after 11 days. Normal values were obtained again 28 days after immunization. The results indicate that the antigen causes an altered relation between input and output of mononuclear cells, mainly lymphocytes.

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Splenic venous blood normally contains a higher concentration of lymphocytes than arterial blood [1, 2], and this may reflect the production of such cells. Release of newly produced cells from the spleen was demonstrated as early as 6 h after labelling with 3H-thymidine [3]. In guinea pigs, this export of lymphocytes from the spleen was shown to comprise both B and T lymphocytes [4]. The lymphocyte release from the spleen may be quantitatively considerable [2, 5, 6]. Primary immunization with sheep red blood cells (SRBC) did not significantly affect the magnitude of this splenic output of cells [7], but an increased number of released cells was recorded after immunization with pertussis vaccine [5]. Release of antigen-specific cells occurred during the secondary response to SRBC [8, 9]. In pigs, Pabst and Pötschick [10] demonstrated increased lymphocyte proliferation in the spleen after secondary immunization with SRBC and migration of newly formed cells to both T and B lymphocyte areas of peripheral lymphoid organs. The present work demonstrates that immunization with the *Salmonella typhi* H antigen produces an inhibited net release of cells from the spleen of guinea pigs.

Male guinea pigs (pigmented, rough-haired strain) weighing 297 ± 14 g (mean $\pm$ SD; $n = 109$) were injected intraperitoneally with 1 ml *S. typhi* H (containing 14×10^9 bacteria per millilitre) from the National Bacteriological Laboratory, Solna, Sweden, and examined 1 ($n = 14$), 3 ($n = 15$), 5 ($n = 14$), 7 ($n = 16$), 9 ($n = 14$), 11 ($n = 16$), 15 ($n = 9$) and 28 days ($n = 11$) later. This vaccine contains a suspension of formalin-killed *S. typhi* of the strain I.S.57, which is rich in the H antigen. The preparation contains 0.4% formalin. Some further animals ($n = 19$) were immunized with a 100 times lower dose of the vaccine, and examined after 7 or 11 days. Controls were either untreated animals (weighing 309 ± 17 g at examination; $n = 12$) or animals (271 ± 19 g; $n = 23$) injected with 0.4% formalin and examined after 11 days. The animals were anaesthetized with pentobarbital sodium (25 mg/kg, intraperitoneally), weighed, and the splenic region was exposed by an incision between the most caudal ribs on the left side. A small incision with microscissors into one

splenic vein allowed the collection of 25 μ l venous blood with a heparinized pipette, and immediately afterwards the same amount of splenic arterial blood was collected by the same procedure. The blood was used for determination of the number of mono- (mainly lymphocytes) and polynuclear cells (granulocytes) per microlitre in a Bürker chamber. Finally, the weight of the spleen was determined. Student's t test was used for the evaluation of a veno-arterial difference in cell number and for the comparison of different groups of animals.

At examination the weight of the animals ranged from 277 g at day 1 to 446 g at day 28.

Immunization resulted in a slight loss of weight (15 g) during the first day. Splenic weight increased after immunization

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Fig. 1. Effect of immunization with *S. typhi* H (14×10^9 bacteria) on splenic weight ( ) and the number of mononuclear leucocytes in splenic arterial ( ) and venous ( ) blood (means $\pm$ SEM).

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Fig. 2. Effect of immunization with *S. typhi* H on the splenic veno-arterial difference in the number of mononuclear cells (means $\pm$ SEM). The level in untreated animals is indicated by horizontal lines (SEM values).

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from 500 mg at day 0 to a maximum of 770 mg on day 11 (fig. 1) and was at that time significantly higher than in control animals injected with formalin ($p < 0.05$). Immunization resulted in lymphocytosis at 5, 9 and 11 days (fig. 1). In contrast, immunization did not cause any significant change in the blood granulocyte level (not shown). Untreated animals had a significant veno-arterial difference of mononuclear cells ($p < 0.02$), indicating a net export of lymphocytes from the spleen (fig. 2). A significant export persisted on day 1 ($p < 0.05$), day 3 ($p < 0.01$) and day 5 ($p < 0.02$). There was then a gradual decrease in veno-arterial difference, an effect which was statistically significant from day 7. Normal values were obtained again 28 days after immunization (fig. 2), as well as in controls injected with formalin. There was no significant difference in polynuclear cell content between splenic venous and arterial blood at any time after immunization nor in untreated animals (not shown). Immunization with a 100

times lower dose of bacteria had the same effect on body weight, but the splenic veno-arterial difference in cell content was not affected nor the splenic weight.

The splenic veno-arterial difference in number of mononuclear cells (lymphocytes) shows the net effect of many interacting processes, such as immigration and homing of cells from other sites and the release of cells, including those produced in the spleen, via efferent venous vessels.

Thus, this parameter mirrors the cellular communication of the spleen with the rest of the organism and in the intact animal. According

to the present results, immunization with S1. typhi totally abolished the splenic veno-arterial mononuclear cell difference. This could have been the result of decreased production of lymphocytes, the increased homing of cells to the spleen, inhibition of cell release or a combination of these. The effect may have been caused by the direct interaction of the antigen with lymphocytes, affecting the migratory behaviour. It may also have been mediated by an indirect mechanism, such as lymphokine release, resembling the inhibited neutrophil migration by lipopolysaccharide (LPS) [11]. A proliferative response of the spleen is a normal component in the reaction to immunization, so the observed effect in cell output is not likely due to decreased cell proliferation. It is not likely a stress-induced steroid-mediated reaction, since an earlier study did not show such an effect of prednisolone in intact animals [12]. One component of the bacterial wall, LPS, has been shown to affect lymphocyte migration within the spleen [13]. However, our own (unpublished) observations on the effect of LPS on splenic lymphocyte release do not indicate that the abolished splenic veno-arterial differences in the present work were mediated by LPS.

There was a fairly good correlation between splenic weight and the level of lymphocytes in arterial blood (fig. 1). However, the comparison of splenic venous and arterial blood shows that this does not reflect an increased output of cells from a hypertrophic spleen. Rather, part of the increase in splenic weight may have resulted from an increased input of cells.

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This is supported to some extent by the simultaneous lack of splenic weight increase and lack of decreased splenic net output of lymphocytes after immunization with the lower dose of antigen. Immunization with endotoxin derived from *Haemophilus influenzae* or *Escherichia coli* causes decreased β -adrenoceptor function in guinea pig splenic lymphocytes [14]. Further, an effect of *Salmonella* on adrenergic mechanisms in the spleen was suggested by studies in mice [15]. The export of lymphocytes from the guinea pig spleen has been shown to be increased by adrenergic stimuli, but the combined adrenergic α - and β -receptor blockade did not abolish the export of cells [16].

What is then the functional implication of the inhibited cell migration? This is difficult to evaluate due to the lack of more detailed information regarding the changed migratory behaviour. Of special interest for the ability of the spleen to contribute to the immune response is the seeding of antigen-specific cells from the spleen to other sites. Although the export of antigen-specific cells may amount to 5,000 cells per milli-litre of blood [8], this is still only a minor part of the total cell content. In addition, the export of such cells may precede the effect seen here on cell traffic in the spleen [8, 17]. Therefore, the present results do not exclude that a release of antigen-specific cells occurred in the α -immunized animals, in spite of the demonstrated inhibition of cell output.

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