Results

Before treatment mean blood pressure was 158·0/98·4 mm Hg supine and 148·7/103·2 mm Hg erect and the mean arterial blood pressure was 118·3 mm Hg (supine and erect) (figure). Blood pressures recorded while subjects were receiving any of the three doses of placebo were not significantly different from those recorded before treatment.

Supine blood pressure was significantly reduced with all three doses of natural progesterone when compared with pretreatment readings. The maximum fall was with 300 mg twice daily, the mean being 19·7 mm Hg systolic and 9·6 mm Hg diastolic. Supine blood pressure was always lower during treatment with progesterone than with placebo, but the difference did not always reach significance (100 mg diastolic, $p < 0.05$; 200 mg mean arterial pressure, $p < 0.05$). Erect blood pressure with progesterone was also reduced when compared with pretreatment readings, the difference reaching significance with 300 mg twice daily in the diastolic and mean arterial blood pressure readings. When compared with placebo, there was a significant reduction in erect diastolic ($p < 0.001$) and mean arterial blood pressures ($p < 0.002$) with the 300 mg dose.

No significant changes were observed in pulse rate or weight during the trial. Two men reported slight light headedness about one hour after ingestion of the two higher doses.

Discussion

Blood concentrations of progesterone in premenopausal women are high in the luteal phase of each ovulatory menstrual cycle but fall to 30% of the follicular phase in postmenopausal women. Men have similarly low blood concentrations of progesterone. The results of this pilot study suggest that natural progesterone produces a significant reduction in blood pressure at doses which give plasma concentrations that are just above luteal phase concentrations.

The physiology of progesterone suggests that its antihypertensive action is peripheral, although an additional central action cannot be excluded. In this study the less predictable reduction of erect blood pressure could have been due to the presumed vasodilation action of natural progesterone being overridden by reflex sympathetic vasoconstrictor activity.

We suggest that progesterone is a "protective" female hormone. The low blood progesterone concentrations present after the menopause could account for the finding that the prevalence of high blood pressure and incidence of cardiovascular disease in women tend to catch up with those in men.[1 3] This property would recommend the use of natural progesterone in combined oral contraceptives instead of synthetic gestagens.

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Impaired antipneumococcal antibody production in patients without spleens

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Abstract

Fifteen splenectomised and 15 normal subjects were studied, in absence of any intentional immunisation, for pokeweed mitogen induced synthesis of antipneumococcal capsular polysaccharide antibodies in vitro by peripheral blood mononuclear cells. Results showed that removal of the spleen had caused a persistent immune deficiency of circulating B cells capable of synthesising IgM antipneumococcal capsular polysaccharide. In vitro synthesis of polyclonal IgM and IgG by peripheral blood mononuclear cells of subjects without spleens was also depressed. These defects were due to an abnormality of the B cell compartment.

These data are evidence of the major role of the spleen in the control and production of a consistent part of pokeweed mitogen responsive circulating B cells and add another facet to the complex immune dysfunction of splenectomised subjects. The findings, moreover, may help in understanding the susceptibility of splenectomised people to pneumococcal sepsis and the delayed and impaired antibody response to pneumococcal vaccine.

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Introduction

Despite the wide range of antibiotics available pneumococcal infections remain a substantial cause of morbidity and mortality.[1] In asplenic children and patients whose spleens have been removed for therapeutic reasons or after trauma overwhelming pneumococcal sepsis and an increased incidence of pneumococcal infections have been described.[2] The association between absence of the spleen and infections has stimulated a great number of clinical and experimental investigations that have shown the relevant role of the spleen in the defence against

bloodborne viral and bacterial infections. Many immune defects have been reported, including loss of mechanical filtration, diminished phagocytic activity, decreased opsonin concentrations, decreased serum IgM concentrations, depressed properdin titres, reduced tuftsin concentrations,[3] altered T helper and T suppressor cell activity,[4 5] and impaired antibody production after intravenously administered antigens and in response to pneumoccocal and meningococcal vaccines.[6-8]

In a previous study we showed that after immunisation with a 14 valent pneumococcal vaccine the spleen has a central role in the control of circulating lymphocyte subsets capable of synthesising antipneumococcal capsular polysaccharide antibodies.[9] We have now studied, in absence of any intentional immunisation, the synthesis of antipneumococcal capsular polysaccharide antibodies and total IgM and IgG in vitro by peripheral blood mononuclear cells of 15 splenectomised and 15 normal subjects. We also evaluated the contribution of B cells to the defects observed.

Subjects and methods

Fifteen splenectomised men aged 22-65 years (mean 42·3 (SE 3·0) years) and 15 male volunteers aged 24-48 years (mean 36·5 (SE 1·6) years) entered the study. Splenectomy had been performed for trauma two months to 23 years previously. No subject had evidence of an immunological or haematological disease, and at the time of study all were in good health. Total lymphocyte counts (mean 3118 (SE 249) $\times 10^9$/l, range 2140-4720 $\times 10^9$/l), and serum IgM (0·71 (0·07) g/l, range 0·33-1·20), IgG (11·98 (0·78) g/l, range 8·00-17·20), and IgA (2·89 (0·21) g/l, range 1·60-3·90) concentrations were obtained in all the patients with splenectomy. Informed consent was obtained from all subjects.

Cell preparations—Blood samples were obtained in the morning after an overnight fast. Peripheral blood mononuclear cells were separated by Ficoll-diatrizoate (LSM; Bionetics) density centrifugation of heparinised blood. Interface cells were removed and washed three times with phosphate buffered saline containing 2% fetal calf serum (KC Biologicals) at room temperature to remove cytophilic immunoglobulin. T enriched (T_e) and B enriched (B_e) cell fractions were separated by density centrifugation of spontaneous rosettes formed by T cells with sheep red blood cells pretreated with 2-aminoethylisothiouronium bromide hydrobromide (Sigma). The T_e cell fractions of both the splenectomised and normal subjects contained more than 90% OKT3 positive cells, fewer than 2% surface membrane immunoglobulin positive cells, and fewer than 2% α-naphthyl-acetate-esterase positive cells. The B_e cell fractions contained 40-55% surface membrane immunoglobulin positive cells, 35-50% α-naphthyl-acetate-esterase positive cells, and fewer than 5% OKT3 positive cells. The relative proportions of T cells, B cells, and monocytes in normal and splenectomised subjects were comparable notwithstanding an enhanced recovery of mononuclear cells per ml blood in splenectomised subjects. Viability in cell fractions exceeded 95% as measured by trypan blue dye exclusion. In co-culture experiments T_e cells were irradiated (30 Gy; 3000 rads) to remove T cell suppressor function.

Culture conditions—Unseparated peripheral blood mononuclear cells (2×10^6) and fractionated and reconstituted combinations of B cells ($0{\cdot}4 \times 10^6$) and irradiated T cells ($0{\cdot}8 \times 10^6$) from splenectomised or control subjects were cultured in Roswell Park Memorial Institute 1640 medium (GIBCO), buffered with sodium bicarbonate, and supplemented with L-glutamine (2 mmol/l; 29·2 mg/100 ml) and penicillin (100×10^3 U/l) and 15% heat inactivated fetal calf serum. Cultures were made up to a volume of 1 ml in 12 × 75 mm plastic tubes (Falcon) in the presence and absence of pokeweed mitogen (GIBCO) at a final concentration of 1/400 vol/vol. All the cultures were done in duplicate. The tubes were incubated at 37°C in a humidified mixture of 5% carbon dioxide and air for 12 days. At the end of the culture period the cells were spun down and supernatants recovered and kept frozen till the time of assay.

Radioimmunoassays—Quantitative radioimmunoassays for IgM and IgG antitype 2 and antitype 3 pneumococcal capsular polysaccharides and for total IgM and IgG were performed as described.[9] The values for IgM and IgG antipneumococcal capsular polysaccharides and for total IgM and IgG were calculated in reference to standard curves obtained by using serial twofold dilutions of a standard human serum (Hoechst Pharma AG). The standard curve for IgM was linear in the range 30·6 and 0·95 μg/l ($r = 0{\cdot}999$) and that for IgG linear in the range 73·8 and 1·15 μg/l ($r = 0{\cdot}997$). Since the affinity between antigens and antibody and between anti-immunoglobulins and immunoglobulins may be different, however, the absolute values for IgM and IgG antipneumococcal capsular polysaccharides must be regarded as indicative only. Titres below 1·0 μg/l were assigned a value of 1·0 μg/l for calculation. IgM and IgG antipneumococcal capsular polysaccharides were not detected in the culture supernatants from unstimulated cultures, while total IgM and IgG values were less than 200 μg/l. Results are presented as the arithmetic means of the duplicate cultures.

Statistical analysis—Student's *t* test for unpaired data was used to assess differences between groups. All values are reported as means and standard error (SE).

Results

After stimulation with pokeweed mitogen peripheral blood mononuclear cells from splenectomised subjects showed a profound inability to secrete antipneumococcal capsular polysaccharide antibodies of the IgM class (table I). By contrast, these were produced by the peripheral blood mononuclear cells of all the normal controls. Antipneumococcal capsular polysaccharide antibodies of the IgG class were detected only occasionally in the culture supernatants of both splenectomised and normal subjects, and no correlation was observed between the values of IgG and IgM antipneumococcal capsular polysaccharides. In addition, in the patients with splenectomy a significant decrease in the synthesis of polyclonal IgM and IgG was detected. No relation was established between the in vitro secretion of specific or polyclonal antibodies and the age of the

TABLE I—*Pokeweed mitogen induced in vitro synthesis of IgM and IgG antitype 2 and antitype 3 pneumococcal capsular polysaccharides (PCP) and of total IgM and IgG in splenectomised subjects*

Case No	Date of splenectomy	IgM antitype 2 PCP (μg/l)	IgM antitype 3 PCP (μg/l)	Total IgM (μg/l)	IgG antitype 2 PCP (μg/l)	IgG antitype 3 PCP (μg/l)	Total IgG (μg/l)
1	1983	3	1	900	4	1	1100
2	1983	1	1	100	1	1	1200
3	1979	9	12	2100	2	2	3200
4	1979	26	4	2200	14	2	2800
5	1976	1	1	1700	1	1	1600
6	1976	7	19	2000	1	1	8400
7	1973	8	3	1100	2	9	2400
8	1970	31	25	4700	1	1	3100
9	1967	1	1	500	1	1	1300
10	1966	7	10	3800	3	1	6600
11	1965	2	3	1600	7	1	5800
12	1964	12	1	200	11	3	4700
13	1964	1	1	300	1	1	1200
14	1961	9	3	200	1	1	1400
15	1960	2	3	700	1	6	2800
Mean (SE)		8·0 (2·4)	5·9 (1·9)	1473 (349)	3·4 (1·1)	2·1 (0·6)	3173 (579)
Controls (n = 15)	Mean (SE)	21·7 (3·5)	14·3 (2·2)	10 434 (2145)	5·1 (1·9)	4·5 (1·7)	7046 (1162)
	Range	8-45	3-30	2500-23 000	1-27	1-16	1500-18 000
p Value		<0·01	<0·01	<0·001	NS	NS	<0·01

NS = Not significant.

TABLE II—*Depressed immunoglobulin production by B cells of splenectomised subjects.* Values are means (SE in parentheses)*

Source of B cells	Source of T cells	IgM antitype 2 PCP† (μg/l)	IgM antitype 3 PCP† (μg/l)	Total IgM (μg/l)	Total IgG (μg/l)
Splenectomised subjects (n = 8)	Splenectomised subjects (n = 8)	7·6 (2·2)	4·0 (1·9)	1850 (235)	4635 (1080)
Splenectomised subjects (n = 8)	Control subjects (n = 8)	9·6 (4·1)	5·1 (2·8)	2173 (696)	4922 (1230)
Control subjects (n = 8)	Control subjects (n = 8)	31·0 (4·3)‡	13·2 (2·4)‡	12 450 (2616)‡	9120 (1832)‡

*B cells of each splenectomised subject ($0·4 \times 10^6$ B cells) cocultured with irradiated T cells ($0·8 \times 10^6$ T cells) from same subject or from control in presence of pokeweed mitogen. B cells of each control subject cultured with irradiated T cells from same control.
†PCP = Pneumococcal capsular polysaccharide.
‡$p < 0·05$ compared with other two groups.

subject, time since splenectomy, total lymphocyte count, or serum immunoglobulin concentration.

To define the role of B cells in the defects observed, allogeneic cocultures were performed. B cells from splenectomised subjects were cultured with autologous or allogeneic T cells irradiated in order to eradicate radiosensitive suppressor activity and optimise helper activity. The synthesis of both specific and polyclonal immunoglobulin by the B cells of splenectomised subjects was clearly impaired and could not be improved by coculture with allogeneic helper T cells (table II).

Discussion

The spleen in the defence mechanism against pneumococcal infections has two immune functions: it is a site of production of specific antibodies and serves as a filter system for opsonised bacteria in the blood stream.[10] This study shows that, in addition, the spleen has a major role in the control of circulating B cells capable of differentiating in vitro into antipneumococcal capsular polysaccharide antibody secreting B cells. Our data show that in the absence of intentional immunisation IgM antipneumococcal capsular polysaccharide antibodies are often missing in the culture supernatants of splenectomised adults whereas they are an invariable feature of in vitro IgM synthesis in control subjects. The existence in normal subjects of circulating B cells able to secrete specific antibodies accords with the presence of type specific pneumococcal antibodies in the serum of non-vaccinated people[6 11] and with the observation that pokeweed mitogen can induce antigen specific IgM synthesis in vitro even in absence of previous contact with the antigen.[12] On the other hand, the abnormal production of antitype 2 and antitype 3 pneumococcal capsular polysaccharide antibodies in splenectomised subjects is not explained by a dilution of functional lymphocytes, as in several cases the synthesis of specific antibodies was completely absent and no correlation was established with the absolute number of peripheral blood lymphocytes. Recently it has been reported that the serum concentration of IgM antitype 2 and antitype 3 pneumococcal capsular polysaccharide antibodies is low in non-vaccinated subjects splenectomised after trauma.[6 11] That observation lends support to our findings even if a correlation between in vitro antibody synthesis and resting in vivo antibody serum concentrations has not been shown.[12 13]

The defective synthesis of specific antibodies in splenectomised subjects is detectable many years after surgery, cannot be ascribed to other pathological conditions (all our subjects were healthy and had had their spleens removed after trauma), and is mainly dependent on B cell dysfunction, as it cannot be improved by adding normal T cells. In addition, it is associated with a more profound and generalised inability of peripheral blood B lymphocytes to differentiate into immunoglobulin secreting cells.[9 14 15]

The importance of the spleen in maturation of B cells is confirmed by numerous experimental data. In mice the spleen is the primary site of specific antibody production against pneumococci.[4] In rabbits and rodents the spleen provides a highly effective environment for the activation of antibody forming precursors and for the production and release of memory B cells that later are found in the peripheral blood and in other lymphoid tissues.[16 17] There are, moreover, indications that the unique microcirculation of the spleen, which allows direct contact between antigens and B lymphocytes, facilitates the immune response to intravenously administered particulate antigens.[3 18] On the other hand, much evidence indicates that the spleen is necessary to mount a rapid and appropriate antibody response against pneumococcal polysaccharides[6] and that IgM antibodies are essential in the defence mechanism against pneumococci.[10]

In conclusion, our findings show that absence of the spleen causes a longlasting B cell defect characterised by a limited capacity of circulating B cells to differentiate into antibody secreting cells. Even if our findings are not the sole explanation for the increased incidence of pneumococcal sepsis in splenectomised subjects, they do show that in these subjects the immune response to pneumococcal capsular polysaccharide is impaired at the cellular level. We suggest that our results may offer a clue to the link between the absence of the spleen, depressed serum antibody response to pneumococcal capsular polysaccharide, delayed and depressed antibody response to pneumococcal vaccine, and limited ability to survive pneumococcal sepsis.

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