was that the spectinomycin resistant PPNG strains from St Mary's Hospital were all isolated after treatment with spectinomycin, whereas those from St Thomas's Hospital were not.

We do not know whether these strains first acquired the ability to produce β-lactamase and then mutated to spectinomycin resistance or whether they represented gonococci with an abnormally high propensity to mutate to spectinomycin resistance that have acquired the 4·4 plus 24·5 megadalton plasmids for β-lactamase production and transfer. Wider screening of gonococci for resistance to spectinomycin may help to answer this question. Our experience so far is that of over 3000 non-β-lactamase producing gonococci tested by the disc diffusion method only six (four from St Mary's Hospital and two from St Thomas's Hospital) were resistant to spectinomycin even though spectinomycin was introduced as first line treatment for gonorrhoea in heterosexuals at St Mary's Hospital in January 1983. In contrast to the situation often seen with spectinomycin resistant PPNG, all six of these spectinomycin resistant non-PPNG were isolated before spectinomycin treatment.

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Role of spleen in immune response to polyvalent pneumococcal vaccine

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Abstract

The immune response of lymphocytes to subcutaneously administered pneumococcal vaccine was studied in five patients without spleens and in five healthy subjects. Seven days after immunisation circulating B cells synthesising IgG antipneumococcal capsular polysaccharides (anti-PCP) appeared in both groups. Twenty one days after vaccination this B cell population had disappeared and a B cell subset which secreted IgM and IgG anti-PCP in the presence of pokeweed mitogen was detected in the normal but not in the splenectomised subjects.

In the splenectomised group polyclonal IgM synthesis induced by pokeweed mitogen was defective.

It was concluded that the early events of the immune response to PCP may be mediated by lymph nodes but that, later, the spleen acquires a central role in producing lymphocyte subsets capable of synthesising specific antibodies and that this might explain the increased sensitivity of splenectomised subjects to pneumococcal infection.

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Introduction

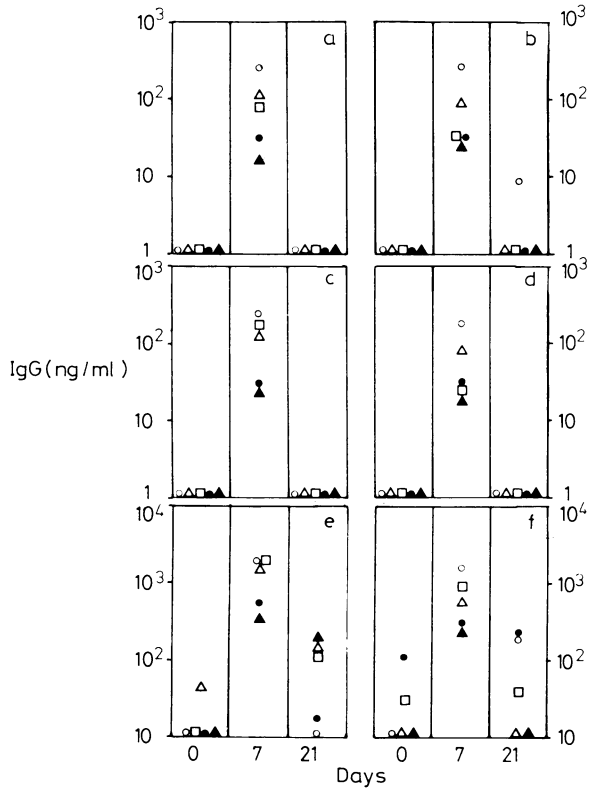
Despite the use of antibiotics pneumococcal infections continue to cause illness and death throughout the world.[1 2] Patients who do not have spleens are particularly at risk[3] and many clinical trials have assessed the efficacy of polyvalent pneumococcal vaccines in these patients. Some studies have shown the effectiveness of vaccination in affording protective concentrations of specific antibodies,[4-7] while others have shown a limited serological response.[8 9]

Experimentally the cellular immune events that occur after immunisation with pneumococcal capsular polysaccharide (PCP) depend on the spleen for the response to the T cell independent antigen.[10 11] We have investigated at different intervals after vaccination in vitro IgM and IgG anti-PCP synthesis by peripheral blood lymphocytes in five splenectomised and five normal subjects to see if the same applies in man.

Patients and methods

Five splenectomised men (mean age 37·4 (SE 2·0) years, range 32-44) and five healthy male volunteers (mean age 33·0 (SE 2·0) years, range 28-39) consented to receive a subcutaneous injection of 0·5 ml polyvalent PCP vaccine (Pneumovax). At the same time they were given a booster intramuscular injection of 20 Lf (limit of flocculation) units soluble tetanus toxoid (Te-Anatoxal; Berna). Splenectomy had been performed for trauma at least three years before.

Blood samples were obtained before and seven and 21 days after immunisation. Mononuclear cells were separated by Ficoll-diatrizoate (LSM; Bionetics) density centrifugation of heparinised blood. The interface cells were removed and washed three times with phosphate buffered saline containing 2% fetal calf serum (KC Biologicals).



In vitro production of IgG anti-type 2 (upper panels: a, b) and anti-type 25 (middle panels: c, d) PCP and of total IgG (lower panels: e, f) by B_e cell fractions from five splenectomised patients (left panels: a, c, e) and from five normal subjects (right panels: b, d, f) immunised with 14 valent pneumococcal vaccine. Cultures set up before and seven and 21 days after immunisation.

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).[12] Some T_e lymphocytes were irradiated (3000 rads) to remove suppressor functions.[13] Either fractionated ($0{\cdot}4 \times 10^6$ B_e cells; $1{\cdot}6 \times 10^6$ T_e cells) or fractionated and reconstituted ($0{\cdot}4 \times 10^6$ B_e cells + $1{\cdot}6 \times 10^6$ T_e cells) mononuclear cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (GIBCO) buffered with sodium bicarbonate supplemented with L-glutamine (2 mmol/l) and penicillin (100×10^3 units/l), streptomycin (100 mg/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 or absence of pokeweed mitogen at a final dilution of 1/400 vol/vol. The tubes were incubated at 37°C in a humidified mixture of 5% CO_2 and air for 12 days. Cultures were fed with 0·2 ml complete fresh medium every four days. At the end of the culture period the cells were spun and supernatants recovered and kept frozen till the time of assay.

Quantitative radioimmunoassays for IgM and IgG anti-PCP and for total IgM and IgG were performed in flexible microtitre plates (Dinatech). Solutions of type 2 and type 25 PCP were maintained at a concentration of 1 g/l in phosphate buffered saline (pH 7·2) at 4°C until use. PCP was covalently bound to poly-L-lysine (molecular weight 30 000-70 000; Sigma) using cyanuric chloride as the coupling agent.[11] A 50 µl sample of a stock solution (50 mg/l) of each PCP or of affinity purified goat antihuman IgM or antihuman IgG antibody (100 mg/l) in phosphate buffered saline was added to the inner wells of microtitre plates and incubated overnight at room temperature in a humidified chamber. The next day each well was washed three times with 1% bovine serum albumin in phosphate buffered saline. A 5% solution was then added and four hours later the microtitre plates were again washed three times. Plates so prepared were stored at 4°C until used.

For determinations of IgM and IgG anti-PCP 50 µl of the culture supernatants and their 1/5 dilutions were added in duplicate to the wells. For total IgM and IgG determinations 50 µl of 10-fold dilutions of the supernatants were added. A standard human serum (Hoechst-Pharma AG) was serially diluted twofold over an IgM range of 980 to 0·95 µg/l and an IgG range of 1180 to 1·15 µg/l. A sample of 50 µl of each dilution of reference serum was added to the wells in duplicate to build up reference standard curves. The plates were incubated overnight at room temperature. The next day the plates were washed three times and each well filled with 50 µl containing 20 ng of affinity purified ^{125}I-goat antihuman IgM antibodies (specific activity 3820 cpm/ng) or ^{125}I-goat antihuman IgG antibodies (specific activity 2340 cpm/ng) in 1% bovine serum albumin in phosphate buffered saline and 0·05% Tween 80. After overnight incubation at room temperature the wells were again washed three times and twice with tap water.

The standard curve for IgM was linear in the range 30·6 and 0·95 µg/l ($r = 0{\cdot}998$) and that for IgG linear in the range 73·8 and 1·15 µg/l ($r = 0{\cdot}995$). The values for IgM and IgG anti-type 2 and anti-type 25 PCP and for total IgM and IgG were calculated in reference to these standard curves. Titres below 1·0 µg/l were assigned a value of 1·0 µg/l for calculation.[8] All samples were run in duplicate at a single time to reduce day to day variations. A suitable dilution of control serum was run in all the microtitre plates to check plate to plate variations that were consistently less than 10%. The specificity of the radioimmunoassays for human IgM and IgG anti-type 2 and anti-type 25 PCP was confirmed by complete inhibition after adding, in the first incubation, the appropriate PCP (500 ng) in soluble form. No binding competition was seen when the other PCP or an unrelated antigen (tetanus toxoid; 500 ng) was added. Student's *t* test for unpaired data was used to assess differences between groups. All values are reported as means and SE.

Results

Before immunisation the B_e cell fractions from splenectomised and normal subjects did not synthesise detectable quantities of IgG anti-PCP (figure). Seven days after immunisation the appearance of cells capable of secreting IgG anti-PCP, without T cell or mitogen requirement, was detected in both groups of subjects (figure). Since the range of IgG anti-PCP values was wide, differences were not shown between the two groups. The ability to synthesise IgG anti-PCP disappeared 21 days after vaccination. Synthesis of polyclonal IgG by unstimulated B_e cells paralleled that of specific antibodies, since it was low before vaccination, increased at seven days, and returned to baseline at 21 days (figure). High concentrations of total IgG at seven days were due to the presence of B cells spontaneously secreting antibodies against the 14 valent pneumococcal vaccine. Over the same time an analogous synthesis of IgM anti-PCP by unstimulated B_e cells could not be detected and changes in the production of polyclonal IgM were not observed.

Twenty one days after immunisation significant differences between splenectomised and normal subjects were detected in the synthesis of IgM and IgG anti-type 2 and anti-type 25 PCP and of polyclonal IgM by mitogen stimulated lymphocyte cultures (tables I and II). Polyclonal stimulation of reconstituted B_e and T_e cell fractions from splenectomised subjects did not induce secretion of measurable amounts of specific IgM or IgG antibodies, and irradiation of T lymphocytes resulted in low amounts of specific antibodies in a minority of patients. In addition, the production of polyclonal IgM was significantly impaired in mitogen stimulated cultures, and this defect was not abolished by irradiation of T cells. This reduced biosynthesis was consistently found whenever cells were activated with pokeweed mitogen. In all but one of the normal subjects pokeweed mitogen stimulated the secretion of specific IgM and IgG antibodies and T cell irradiation produced a conspicuous increase in titre of IgM and IgG anti-PCP (tables I and II).

Discussion

The central role of the spleen in the body's immune response is exemplified by the increased risk of serious pyogenic infections in people without a spleen or with a non-functioning spleen. After in vivo immunisation with pneumococcal vaccine two phases in the immune response may be distinguished. The first is characterised by the appearance in the circulation of B cells secreting antigen specific antibodies, and the second by the presence of cells that secrete antigen specific antibodies only after polyclonal stimulation.[15] We have shown that the early phase develops consists mainly of IgM plaque forming spleen cells; splenectomy abolishes most of the early lymphocyte response. The fact that seven days after immunisation there was no difference in the response of splenectomised and normal subjects may have been due to the different route of immunisation. Patients without spleens have a deficient primary antibody response to intravenously injected antigens,[5] while subcutaneous administration results in low normal concentrations of antibodies.[4 5]

Twenty one days after vaccination cells autonomously secreting IgG anti-PCP are no longer detectable. At the same time lymphocytes of splenectomised subjects cannot be induced to

TABLE I—*Pokeweed mitogen induced in vitro synthesis of IgM anti-type 2 and anti-type 25 PCP and total IgM 21 days after immunisation*

Subject No	IgM anti-type 2 PCP (μg/l)		IgM anti-type 25 PCP (μg/l)		Total IgM (μg/l)	
	B cells + T cells	B cells + irradiated T cells	B cells + T cells	B cells + irradiated T cells	B cells + T cells	B cells + irradiated T cells
			Splenectomised patients			
1	1·0	16·0	1·0	6·0	210	1200
2	1·0	1·0	1·0	1·0	170	1400
3	1·0	12·0	1·0	18·0	420	1300
4	1·0	2·0	1·0	20·0	300	1700
5	1·0	1·0	2·0	2·0	800	2200
Mean (SE)	1·0 (0)	6·4 (3·2)	1·2 (0·2)	9·4 (4·0)	380 (113)	1560 (180)
			Controls			
6	20·0	83·0	15·0	88·0	5000	9400
7	19·0	98·0	27·0	134·0	7400	9100
8	20·0	105·0	22·0	57·0	4000	7200
9	10·0	20·0	12·0	26·0	3100	4700
10	1·0	6·5	3·5	9·0	2600	8600
Mean (SE)	14·0 (3·8)	62·5 (20·5)	15·9 (4·1)	62·8 (22·3)	4420 (850)	7800 (861)
p Value	<0·01	<0·05	<0·01	<0·05	<0·01	<0·01

PCP = Pneumococcal capsular polysaccharide.

TABLE II—*Pokeweed mitogen induced in vitro synthesis of IgG anti-type 2 and anti-type 25 PCP and total IgG 21 days after immunisation*

Subject No	IgG anti-type 2 PCP (μg/l)		IgG anti-type 25 PCP (μg/l)		Total IgG (μg/l)	
	B cells + T cells	B cells + irradiated T cells	B cells + T cells	B cells + irradiated T cells	B cells + T cells	B cells + irradiated T cells
			Splenectomised patients			
1	1·0	4·0	1·0	9·0	2550	11000
2	1·0	1·0	1·0	1·0	1050	8200
3	1·0	4·0	1·0	1·0	2900	9200
4	1·0	1·0	1·0	1·0	1300	3000
5	1·0	1·0	1·0	1·0	3450	10700
Mean (SE)	1·0 (0)	2·2 (0·7)	1·0 (0)	2·6 (1·6)	2250 (463)	8420 (1447)
			Controls			
6	2·5	21·0	2·5	40·0	9700	13400
7	2·0	12·0	4·0	20·0	6200	10500
8	3·0	19·0	4·0	41·0	3220	11000
9	3·0	10·0	1·0	16·0	1820	4100
10	1·0	1·0	1·0	1·0	2600	8700
Mean (SE)	2·3 (0·4)	12·6 (3·6)	2·5 (0·7)	23·6 (7·6)	4708 (1451)	9540 (1553)
p Value	<0·01	<0·05	NS	<0·05	NS	NS

NS = Not significant.

in both splenectomised and normal subjects, while the second is detectable only in subjects with a functional spleen. At the same time a defect in the mitogen dependent polyclonal IgM antibody response may be shown in splenectomised patients.

The transient appearance of such B cells after antigenic stimulation has been reported both in animals and in man. In animals antigen stimulated lymph nodes release into the efferent lymph and circulation lymphocytes secreting antigen specific antibodies.[16] Similarly B cells capable of synthesising antigen specific antibodies of IgG class in the absence of T cells and mitogen are present in the circulation of normal people from five days until 14 days after immunisation.[15]

In mice the spleen is the primary site of formation of antibody after intraperitoneal immunisation with PCP[17] and the response synthesise anti-PCP antibodies, and synthesis of polyclonal IgM on stimulation with pokeweed mitogen is defective.

Hence it seems that in the early phase of the immune response the lymph nodes can replace the spleen in releasing cells spontaneously secreting antigen specific IgG antibodies. In the second phase the spleen is the main organ responsible for synthesis of anti-PCP antibodies and may contribute to the particular sensitivity of splenectomised subjects to pneumococcal infections.

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Neutropenia due to β lactamine antibodies

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Abstract

Neutropenia developed in 13 patients during treatment with β lactamines. The time of onset ranged from eight to 27 days after beginning treatment and occurred with doses as low as 40 mg/24 hours. Concomitant symptoms were eosinophilia, rashes, and fever. Leucoagglutinins were detected in eight out of nine patients by the microleucoagglutination technique.

Clinical and serological findings suggested that neutrophils become sensitised as a result of absorption on the cell membrane of drug-antibody immune complexes. An immune mediated pathogenesis for neutropenia induced by β lactamine seems highly probable.

Introduction

Neutropenia induced by β lactamine has been reported in about 150 patients since 1960.[1 2] Symptoms are never severe, and the neutrophil count quickly returns to normal once treatment is stopped. The basic pathogenic mechanism of the complication is not fully understood, but most reports postulate either a toxic effect or dose related damage[3-7]; an immunologically mediated mechanism has rarely been implicated[8 9] owing to the absence of demonstrable drug dependent leucoagglutinins.[10]

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We report 13 patients who developed neutropenia after receiving intravenous or oral β lactamines. Leucoagglutinins were detected in eight out of nine patients tested.

Patients

Table I summarises the 13 cases of neutropenia, which occurred during treatment with penicillin, oxacillin, amoxycillin, ampicillin, cefoperazone, cefotaxime, and ceftazidime. These β lactamines were administered either intravenously (10 cases) or by mouth (three cases) at a dose of 40-250 mg/kg/24 hours for severe sepsis. None of the patients had a history of allergy to penicillin. Other medications administered concomitantly varied, apart from an associated aminoglucoside (streptomycin, gentamicin, or amikacin). We excluded these as offending agents, since they were continued during the episode of leucopenia. In case 10 neutropenia was noted for the first time after oxacillin. Recovery was rapid after withdrawal of this drug, and the patient was given cephazolin. The neutrophil count fell again (to 88×10^6/l) and rapidly returned to normal once the drug was stopped.

Neutropenia was observed as early as day 8 after starting treatment in one patient, the longest latent period being 27 days. The mean time of onset after starting treatment was 19 days. Neutrophil counts ranged from 24 to 1885×10^6/l. Total white cell count was less than 3300×10^6/l in all but one patient, in whom there was a dissociation between absolute neutrophil count (80×10^6/l) and leucocyte count (8000×10^6/l). There was no depression in red blood cell series.

Associated eosinophilia (> 400×10^6/l) was observed in six patients, thrombocytopenia in one, and blood myelocytosis in two. A transient generalised rash occurred in one patient and fever in three. Renal and hepatic function remained normal in all patients. The neutrophil count returned to normal within one to six days after stopping the β lactamines alone.

One patient (case 13) consented to receive a low dose of the same β lactamine (amoxycillin 500 mg by mouth) after her white cell count had returned to normal. This patient experienced a recurrence of the leucopenia, beginning within the next 24 hours.

The bone marrow was examined in six patients. The results showed