Changes in intravascular complement, kininogen, and histamine during Jarisch-Herxheimer reaction in secondary syphilis

CLIVE LOVEDAY AND J S BINGHAM

From the Department of Genitourinary Medicine, Middlesex Hospital, London

SUMMARY Nine patients with secondary syphilis and one control subject were observed for eight hours after the administration of penicillin. Serial clinical observations were made, and blood samples were obtained for the analysis of complement, histamine, and kininogen. Six patients showed Jarisch-Herxheimer reactions, the intensities of which were found to parallel certain changes in activity of complement and concentrations of histamine and kininogen.

Results were analysed statistically. Significant falls were seen in: total haemolytic complement (CH_{50}), C1 inhibitor, C3, functional (haemolytic) C4, and to a lesser extent total C4. Split products of C3 were shown in five of the six patients who had a reaction. There was no change in total glycine rich β -glycoprotein (GBG) or glycine rich γ -glycoprotein (GGG) or evidence of conversion of GBG to GGG. Plasma kininogen concentrations fell and plasma histamine concentrations rose appreciably before and during the clinical phase of the reaction.

These results are discussed in relation to the clinical features and possible pathogenic mechanisms of the Jarisch-Herxheimer reaction.

Introduction

The Jarisch-Herxheimer reaction occurs after the initial adequate treatment of syphilis^{1,2} and other spirochaetal and bacterial infections.³ In syphilis the reaction is characterised by fever, malaise, sweating, anorexia, myalgia, arthralgia, sore throat, headache, exacerbation of the local lesions, increase in pulse rate, and associated blood pressure changes. It starts within 3-4 hours, is greatest at 6-7 hours, and resolves within 24 hours of initial treatment. It may occur with both antibiotic and non-antibiotic treatment.³

The mechanism of the reaction has yet to be fully explained; Bryceson⁴ summarised three main theories. The first is that spirochaetes and bacteria release an endotoxin that acts directly to produce the main features of the reaction. The second is that sudden death of spirochaetes is followed by massive phagocytosis and release of leucocyte pyrogen, which produces the clinical features of the reaction. The third possibility is that antigenic material is released after the administration of treponemocidal drugs, which results in the formation of immune complexes.

In each case complement and related systems might contribute to the reaction.

Loveday⁵ and Fulford *et al*⁶ have shown falls in the early components of the classic pathway of the complement system, which paralleled the severity of the proceeding Jarisch-Herxheimer reaction. The numbers of patients in these studies were insufficient to be analysed statistically. An early reduction in plasma kininogen, which reflects pronounced formation of kinin, has recently been reported in two patients with early secondary syphilis who had striking Jarisch-Herxheimer reactions.⁷ A scheme for the activation of complement, the formation of kinin and other inflammatory mediators, and the possible relation to the clinical features was proposed by these workers.

We here report changes in intravascular histamine, kininogen, and complement (including components specific to the classic and alternate pathways) in nine patients with early secondary syphilis and one control subject during eight hours after the initial therapeutic dose of an antibiotic.

Patients and methods

Ten people were examined, nine patients (Nos 1-4 and 6-10) with early secondary syphilis and one control subject (No 5). The nine patients with early

Address for reprints: Dr J S Bingham, Director, James Pringle House, Middlesex Hospital, London W1N 8AA

Accepted for publication 23 April 1984

secondary syphilis had a history of a typical rash and minimal or no lymphadenopathy. The control subject was a 30 year old member of staff with no history of sexually transmitted disease. All 10 subjects were given an intramuscular injection of 600 000 U procaine penicillin (Distaquine suspension) intramuscularly.

The patients were observed for at least eight hours. Pretreatment observations of temperature, pulse, blood pressure (lying and standing), and clinical features were recorded, and blood samples were taken. Treatment was given and repeated observations and blood samples were taken at ½, 1, 1½, 2, 3, 4, 6, and 8 hours. Each sample was divided into three parts for serum, heparinised plasma (10 ml blood/100 units of heparin), and for plasma with edetic acid. Contact with glass was avoided; samples were separated within 30 minutes, divided into aliquots in plastic tubes, and frozen at -75°C.

COMPLEMENT ASSAYS

All samples were defrosted at room temperature and then stored at 0°C. Aliquots of normal pooled human serum or plasma were used as controls when appropriate. Total haemolytic complement was measured at 50% haemolytic units (CH₅₀) using the method of Osler *et al*⁸ except that one fifth volumes were used. Component C1 esterase inhibitor, C4, C3, and glycine rich β -glycoprotein (GBG) were measured by radial immunodiffusion in 2% agarose containing the appropriate antiserum.⁹ Component C4 was also assayed by a plate haemolytic method estimating only functional C4.⁵

The conversion of preactive C3 (β 1c) to the electrophoretically faster breakdown products C3i (β 1a) was assessed using two dimensional crossed immunoelectrophoresis.¹⁰ The optimum separation of C3 and C3i was achieved in gels consisting of one part 2% agar and three parts 2% agarose.

Conversion of GBG (factor B) to glycine rich γ -glycoprotein (GGG, factor B) was examined by immunoelectrophoresis in 2% agarose gel against anti-GBG/GGG immunoglobulin. The buffers used for immunodiffusion and electrophoresis contained 0.01 mol/l edetic acid. After the completion of immunodiffusion or electrophoresis, plates were washed overnight in 0.9% saline, dried, and then stained with 0.25% Coomassie brilliant blue (BDH Ltd, Poole, UK).

KININOGEN CONCENTRATIONS

Assays of plasma kininogen concentrations were expressed as μ mol/l bradykinin released from plasma kininogen by trypsin. The kinin released was assayed on the isolated rat uterus.¹¹

HISTAMINE CONCENTRATIONS

Plasma histamine concentrations were estimated by a fluorimetric technique described by Lorenz *et al*¹²

IMMUNOGLOBULIN CONCENTRATIONS

Concentrations of IgG, IgM, and IgA were estimated using standard radial immunodiffusion as described previously.⁵ The results were expressed as a percentage of normal human serum.

Results

CLINICAL CHANGES DURING JARISCH-HERXHEIMER REACTION

The nine patients (Nos 1-4 and 6-10) showed variable degrees of clinical change after the initial dose of penicillin; the control subject (No 5) showed no evidence of a reaction. Patients 9 and 10 had severe Jarisch-Herxheimer reactions starting at three hours, continuing until at least eight hours, and disappearing by 24 hours. These patients complained of headache, generalised myalgia and arthralgia, anorexia and nausea, and episodes of shivering and feeling faint; there was a noticeable increase in their pre-existing rashes. Temperatures of both patients rose to about 39°C at the 6-8 hour interval; the resting pulse rates increased from 76 and 72 beats/minute to 104 and 100 beats/minute respectively at 6-8 hours after treatment; and appreciable falls in blood pressure were noted in both patients.

Patients 1, 2, and 3 had moderate reactions starting at four to six hours and improving by eight hours after treatment. They described headaches, nausea, generalised myalgia and arthralgia, shivering,

FIG 1 Changes in mean (SE) body temperature in groups I and II during eight hours after treatment. (Figures in parentheses indicate significance of temperatures compared with those before treatment.)

and in one case (No 2) feeling faint. In all three a rash appeared between three and six hours, and in two it began to fade by eight hours after treatment. Pulse rates increased to between 90 and 100 beats/minute and blood pressure changes were also less pronounced than those seen in the severe Jarisch-Herxheimer reaction. Patient 4 had a slight reaction with a rise in temperature to 37.4°C , a slight rash, nausea, and malaise for only a short period. Patients 6, 7, and 8 showed no objective or subjective evidence of a reaction.

For the purpose of illustrating results the patients were divided into two groups: those having severe (Nos 9 and 10) or moderate (Nos 1, 2, and 3) reactions (group I), and those having no reaction (Nos 6, 7, and 8) (group II). Results were analysed statistically using the Student's *t* test. Figure 1 compares the changes in temperature of the two groups.

CHANGES IN INTRAVASCULAR COMPLEMENT DURING JARISCH-HERXHEIMER REACTION Classic pathway

Figure 2(a) shows that a fall in total haemolytic complement (CH_{50}) was seen in group I and was most pronounced in patients having a severe reaction and least in patient 4, who had a slight reaction; group II and the control subject showed no fall in total haemolytic complement. The fall in CH_{50} preceded the onset of the clinical features and began 1-2 hours after treatment. It persisted throughout the course of the reaction, and was still appreciably reduced after 4-6 hours. Figures 2(b) and (d) show that concentrations of C1 inhibitor and C3 were appreciably reduced in group I 1-3 hours after treatment, and evidence suggests that concentrations were low but not significant for a longer time. Figure 2(c) shows that C4, measured by the sensitive haemolytic technique (which measures only functional C4),

FIG 2 Mean (SE) changes in (a) total haemolytic complement, (b) C1 inhibitor, (c) functional (haemolytic) C4, and (d) C3 in groups I and II during eight hours after treatment. (Figures in parentheses indicate significance of amounts compared with those before.)

showed a noticeable fall starting one hour after treatment and persisting until eight hours after treatment. The less sensitive estimation of total C4 using radial immunodiffusion (which measures both functional and consumed products of C4) showed less pronounced falls. No changes in CH₅₀, C1 inhibitor, haemolytic C4, or C3 were seen in patients from group II or in the control subject.

Generation of split products of C3, as shown by two dimensional immunoelectrophoresis, occurred in four of the five patients in group I. The table shows that patients who had a severe reaction generated most split products of C3. In each case C3 activation was seen 1-3 hours after treatment when consumption of C3 was greatest. Formation of split products of C3 was not seen in patients from group II or in the control subject.

Alternate pathway

Total GBG and the conversion of GBG to GGG did not change appreciably in any patients or the control subject.

CHANGES IN CONCENTRATIONS OF INTRAVASCULAR HISTAMINE DURING JARISCH-HERXHEIMER REACTION

Figure 3(a) shows an appreciable rise in plasma histamine concentrations in group I starting half an hour after treatment and persisting throughout the eight hours. In patient 9 plasma histamine concentrations increased from 0.6 µg/l to 9.0 µg/l, and in patient 10 from 0.45 µg/l to 8.0 µg/l. Patients 1, 2, and 3 showed a shorter duration of rise or a less noticeable peak in plasma histamine concentrations: (1.0-10.0 µg/l, 1.0-5.7 µg/l, and 0.6-8.0 µg/l respectively), or both. Patients in group II and the control subject showed no change in intravascular histamine concentrations throughout the eight hours.

CHANGES IN CONCENTRATIONS OF INTRAVASCULAR KININOGEN DURING JARISCH-HERXHEIMER REACTION

Figure 3(b) shows an appreciable fall in intravascular kininogen concentrations in group I, which was greatest 1½-4 hours after treatment and had returned to normal by eight hours. In patient 9 the fall was 76% (from 4.2 to 1.1 x mol equivalents of bradykinin/litre of plasma) and in patient 10 it was 86% (from 3.7 to 0.5 µmol equivalents of bradykinin/litre of plasma); both concentrations had returned to normal in eight hours. Similar but less pronounced falls in plasma kininogen concentrations occurred in patients 1 (62%), 2 (39%), and 3 (33%). Patients in group II and the control subject showed no fall in intravascular kininogen concentrations.

CHANGES IN CONCENTRATIONS OF IMMUNOGLOBULINS DURING JARISCH-HERXHEIMER REACTION

Concentrations of IgG, IgM, and IgA did not change appreciably in group I, group II, or the control subject throughout the eight hours.

Discussion

This study shows that during the Jarisch-Herxheimer reaction in secondary syphilis there is appreciable activation of early components of the classic pathway of the complement system, generation of clinically appreciable amounts of histamine, and activation of the kinin forming system.

The Jarisch-Herxheimer reaction was seen in varying degrees of severity in six of the nine patients investigated. A previous report has described the reaction as an "all or none" phenomenon⁴; our results show a variable severity of the reaction assessed by symptoms and clinical signs. A pre-

TABLE Summary of pretreatment and maximum concentrations of intravascular complement

Case No	Severity of Jarisch-Herxheimer reaction	CH ₅₀ (units/ml)		C1 Inh (% NHS)		Total C4 (mg/l)		Functional C4 (% NHS)		C3 (mg/l)		GBG (% NHS)		Split products*: β1c-β1a		
		Pre	Max	Pre	Max	Pre	Max	Pre	Max	Pre	Max	Pre	Max	β1c	β1a	GBG-GGG
1	Moderate	93	62	96	70	418	230	66	24	1400	1100	45	50	+	+	0
2	Moderate	96	46	128	83	240	170	70	23	1400	1080	120	110	+	+	0
3	Moderate	130	102	68	58	495	360	50	26	1000	820	70	66	0	0	0
4	Slight	60	50	85	80	320	240	56	30	1400	1200	68	64	±	0	0
6	Nil	91	79	101	95	320	300	46	42	1030	1000	51	47	0	0	0
7	Nil	94	101	88	85	470	500	100	94	1250	1350	64	58	0	0	0
8	Nil	120	130	140	145	185	175	128	110	1400	1350	89	84	0	0	0
9	Severe	190	80	95	40	605	460	170	43	1420	950	109	103	+	+	+
10	Severe	107	51	120	58	345	230	120	51	1000	740	80	84	+	+	+
5	Nil	80	76	90	88	340	350	110	112	1060	1030	82	79	0	0	0

CH₅₀ = Total haemolytic complement measured at 50% haemolytic units; C1 inh = component C1 esterase inhibitor; GBG = glycine rich β-glycoprotein; NHS = normal human serum.

*Generation of split products scored on scale from 0 (nil) to + + + + (total conversion of preactive to inactive form); β1c = preactive component C3; β1a = component C3i; GGG = glycine rich γ-glycoprotein.

FIG 3 Mean (SE) changes in concentrations of (a) plasma histamine and (b) plasma kininogen (bradykinin equivalent) in groups I and II during eight hours after initial treatment. (Figures in parentheses indicate significance of concentrations compared with those before treatment.)

existing macular rash was potentiated during the reaction, while patients with no rash developed one: in the latter case the rash was fading at eight hours and had disappeared by 24 hours after treatment. These findings are in keeping with the suggestions that the skin, subcutaneous, and other extravascular tissues are important sites of activity during the Jarisch-Herxheimer reaction in syphilis.^{6,7}

Appreciable falls in total activity of haemolytic complement and associated falls in C1 inhibitor, C3, and functional C4, which are early components of the classic pathway, paralleled the intensity of the subsequent Jarisch-Herxheimer reaction and confirmed the findings of other studies using single or small numbers of patients.^{3,4,6} In addition, these falls persisted, which suggests that complement may be used not only in the initial phase but also during the course of the clinical reaction. It is unlikely that complement changes are due to non-specific extravasation of plasma proteins, as plasma concentra-

tions of IgG, IgM, and IgA remained unchanged throughout the period of investigation. Split products of C3 in the plasma, a sensitive test for complement activation, appeared in five out of six patients who had a reaction.

Treponemes may be predominantly extravascular in lymph nodes and skin lesions in secondary syphilis.^{6,7} The extravascular site would be important for complex formation and complement activation, and changes in plasma complement activity and individual plasma complement components would only reflect consumption at these sites. Consistent with this hypothesis is the fact that immune complexes found in the plasma of patients with secondary syphilis vary little before and after the Jarisch-Herxheimer reaction.^{13,14} Lipopolysaccharide and possibly endotoxin released during the Jarisch-Herxheimer reaction in syphilis¹⁵ may activate the alternate pathway¹⁶ but remain undetected in the plasma because of the extravascular site of the reaction.

Interactions between complement and other plasma enzyme systems are multiple.¹⁷ A significant fall in plasma kininogen (the precursor of plasma kinin forming enzymes) may be activated by immune complexes direct or through the Hageman factor. A similar process has been shown in vitro¹⁸ and in vivo in trypanosomiasis of cattle.¹⁹ In addition, lysosomal enzymes released from leucocytes ingesting treponemal debris or recruited by chemotactic factors generated by complement activation would also activate the kinin forming system.²⁰

The appreciable rises in plasma histamine achieved concentrations sufficient to account for at least some of the signs and symptoms occurring during the Jarisch-Herxheimer reaction.²¹ Generation of anaphylotoxins (C3a and C5a), which release histamine from mast cells during complement activation,^{22,23} could give rise to these noticeable increases in plasma (and presumably tissue) histamine concentrations.

Results presented here partly confirm the scheme proposed by Loveday⁵ and Fulford *et al*⁶ suggesting the possible importance of activation of complement, kinin formation, and histamine release in relation to the clinical features of the Jarisch-Herxheimer reaction. Work is in progress to clarify further the role of complement and inflammatory mediators.

References

1. Jarisch A. Therapeutische versuche bei syphilis. *Wien Med Wochenschr* 1895; **45**: 721-4.
2. Herxheimer K, Krause I. Über eine bei syphilitischen vorkommende quicksilberreaktion. *Dtsch Med Wochenschr* 1902; **28**: 895-7.
3. Bryceon ADM. Immune complexes and the Jarisch-Herxheimer reaction. In: Bonomo L, Turk JL, eds. *Proceedings of the international symposium on immune complex diseases*. Milan: Carb Erba Foundation, 1970; 110.

4. Bryceson ADM. Clinical pathology of the Jarisch-Herxheimer reaction. *J Infect Dis* 1976; **133**:696-704.
5. Loveday C. *Blood changes in inflammation and related processes*. London: Council for Academic Awards, 1977. PhD thesis.
6. Fulford KWM, Johnson N, Loveday C, Storey J, Tedder RS. Changes in intravascular complement and antitreponemal antibody titres preceding the Jarisch-Herxheimer reaction in secondary syphilis. *Clin Exp Immunol* 1976; **24**:483-91.
7. Loveday C, Bingham JS. Changes in intravascular kininogen during the Jarisch-Herxheimer reaction in secondary syphilis. *British Journal of Venereal Diseases* 1982; **58**:408-9.
8. Osler AG, Strauss JH, Mayer MM. Diagnostic complement fixation 1 A method. *American Journal of Syphilis, Gonorrhea and Venereal Disease* 1952; **36**:140-54.
9. Mancini G, Carbonara AD, Hermans JF. Immunochemical quantitation of antigen by single radial immunodiffusion. *Immunochemistry* 1965; **2**:235-54.
10. Laurell CB. Antigen-antibody crossed electrophoresis. *Anal Biochem* 1965; **10**:358-61.
11. Eisen V, Loveday C. In vivo effects of cellulose sulphate on plasma kininogen, complement and inflammation. *Br J Pharmacol* 1971; **42**:383-91.
12. Lorenz W, Reimann HJ, Barth H, *et al*. A sensitive and specific method for determination of histamine in human whole blood and plasma. *Hoppe Seylers Z Physiol Chem* 1972; **353**:911-20.
13. Sjølling J, Sjølling K, Jakobsen KU, From E. Circulating immune complexes in syphilis. *Acta Derm Venereol (Stockh)* 1978; **58**:263-7.
14. Engel S, Diezel W. Persistent serum immune complexes in syphilis. *British Journal of Venereal Diseases* 1980; **56**:221-2.
15. Gelfand JA, Elin RJ, Berry FW, Frank MM. Endotoxemia associated with the Jarisch-Herxheimer reaction. *N Engl J Med* 1976; **295**:211-3.
16. Frank MM, May EJ, Kane MA. Contributions of the classical and alternate complement pathways to the biological effects of endotoxin. *J Infect Dis* 1973; **128S**:176-81.
17. Ruddy S, Gigli I, Austen KF. The complement system of man (first part of four parts). *N Engl J Med* 1972; **287**:489-95.
18. Movat HZ, Di Lorenzo NL. Activation of plasma kinin system by antigen-antibody aggregates. I. Generation of permeability factor in guinea pig serum. *Lab Invest* 1968; **19**:187-200.
19. Boreham PFL. Immune reactions and kinin formation in chronic trypanosomiasis. *Br J Pharmacol* 1968; **32**:493-504.
20. Eisen V. Formation and function of kinins. The immunochemistry and biochemistry of connective tissue and its disease states. *Rheumatology* 1970; **3**:103-68.
21. Lorenz W. Histamine release in man. *Agents Actions* 1975; **5**:402-16.
22. Dias de Silva W, Lepow IH. Complement as a mediator of inflammation. II. Biological properties of anaphylatoxin prepared with purified components of human complement. *J Exp Med* 1967; **125**:921-46.
23. Cochrane CG, Muller-Eberhard HJ. The derivation of two distinct anaphylatoxin activities from the third and fifth components of human complement. *J Exp Med* 1968; **127**:371-86.