2. Greco FA, Oldham RK. Current concepts in cancer. Small-cell lung cancer. N Engl J Med 1979; 301:355-8.
3. Greco FA, Einhorn LH, Richardson RL, Oldham RK. Small cell lung cancer: progress and perspectives. Semin Oncol 1978; 5:323-35.
4. Hansen HH, Elliott JA. Patterns of failure in small cell lung cancer: implications for therapy. Recent Results Cancer Res 1984; 92:43-57.
5. Maurer LH, Tulloh M, Weiss RB, et al. A randomized combined modality trial in small cell carcinoma of the lung: comparison of combination chemotherapy-radiation therapy versus cyclophosphamide-radiation therapy effects of maintenance chemotherapy and prophylactic whole brain irradiation. Cancer 1980; 45:30-9.
6. Bunn PA Jr, Lichter AS, Glatstein E, Minna JD. Results of recent studies in small cell bronchogenic carcinoma and prospects for future studies. In: Greco FA, Oldham RK, Bunn PA Jr, eds. Small cell lung cancer. New York: Grune & Stratton, 1981:413-46.
7. Morstyn G, Ihde DC, Lichter AS, et al. Small cell lung cancer 1973–1983: early progress and recent obstacles. Int J Radiat Oncol Biol Phys 1984; 10:515-39.
8. Maurer LH, Pajak T, Eaton W, et al. Combined modality therapy with radiotherapy, chemotherapy, and immunotherapy in limited small-cell carcinoma of the lung: a phase III Cancer and Leukemia Group B study. J Clin Oncol 1985; 3:969-76.
9. Kent CH, Brereton HD, Johnson RE. "Total" therapy for oat cell carcinoma of the lung. Int J Radiat Oncol Biol Phys 1977; 2:427-32.
10. Aisner J, Whitacre M, VanEcho DA, Wesley M, Wiernik PH. Doxorubicin, cyclophosphamide, and VP16-213 (ACE) in the treatment of small cell lung cancer. Cancer Chemother Pharmacol 1982; 7:187-93.
11. The World Health Organization histological typing of lung tumours: second edition. Am J Clin Pathol 1982; 77:123-36.
12. Maurer LH, Pajak TF. Prognostic factors in small cell carcinoma of the lung: a Cancer and Leukemia Group B study. Cancer Treat Rep 1981; 65:767-74.
13. Armitage P. Statistical methods in medical research. Oxford: Blackwell, 1971.
14. Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observations of each patient. II. Analysis and examples. Br J Cancer 1977; 35:1-39.
15. Cohen MH. Is thoracic radiation therapy necessary for patients with limited-stage small cell lung cancer? No. Cancer Treat Rep 1983; 67:217-21.
16. Cox JD. Combined modality treatment of small cell carcinoma of the lung. In: Wiernik PH, ed. Controversies in oncology. New York: John Wiley, 1982:139-53.
17. Byhardt RW, Cox JD. Is chest radiotherapy necessary in any or all patients with small cell carcinoma of the lung? Yes. Cancer Treat Rep 1983; 67:209-15.
18. Aisner J. Is combined modality treatment of small cell carcinoma of the lung necessary? In: Wiernik PH, ed. Controversies in oncology. New York: John Wiley, 1982:155-73.
19. Perez CA, Einhorn L, Oldham RK, et al. Randomized trial of radiotherapy to the thorax in limited small-cell carcinoma of the lung treated with multiagent chemotherapy and elective brain irradiation: a preliminary report. J Clin Oncol 1984; 2:1200-8.
20. Salazar OM, Creech RH. "The state of the art" toward defining the role of radiation therapy in the management of small cell bronchogenic carcinoma. Int J Radiat Oncol Biol Phys 1980; 6:1103-17.

ABSENCE OF HEPATITIS AFTER TREATMENT WITH A PASTEURIZED FACTOR VIII CONCENTRATE IN PATIENTS WITH HEMOPHILIA AND NO PREVIOUS TRANSFUSIONS

K. Schimpf, M.D., P.M. Mannucci, M.D., W. Kreutz, M.D., H.H. Brackmann, M.D., G. Auerswald, M.D., N. Ciavarella, M.D., J. Mösseler, M.D., V. DeRosa, M.D., B. Kraus, M.D., Ch. Brueckmann, M.D., G. Manguso, M.D., U. Mittler, M.D., F. Haschke, M.D., and M. Morfini, M.D.

Abstract Post-transfusion hepatitis is frequent among patients with hemophilia who are treated with concentrated factor VIII prepared from pooled plasma, especially if it is obtained from paid donors. In 26 patients with hemophilia A or von Willebrand's disease who had not been treated with blood or any blood product and hence were highly susceptible to the development of post-transfusion hepatitis, we infused 32 batches of a factor VIII concentrate that had been produced from large pools of human plasma (collected from paid plasmapheresis donors) and then heated in solution at 60°C for 10 hours before final lyophilization. Patients were examined clinically and serologically over a period of 12 months after the first infusion of the pasteurized concentrate. Neither hepatitis nor serologic signs of other viral infections were observed. The hemostatic effectiveness of the concentrate appeared to be satisfactory relative to untreated concentrates. (N Engl J Med 1987; 316:918-22.)

POST-TRANSFUSION hepatitis is common among patients with hemophilia who are treated with commercial factor VIII concentrates manufactured from large pools of plasma from paid donors recruited mostly in plasmapheresis centers in the United States.[1-4] In 1982 hepatitis B virus markers were found in all patients treated with annual doses of 10,000 or more factor VIII units,[1] although the risk of new infections should now be reduced by screening of donors and vaccination of patients. Similarly, there was a 100 percent prevalence of non-A, non-B hepatitis, diagnosed by the presence of raised aminotransferase levels after infusion and by the exclusion of other causes of hepatitis, in patients who were at high risk of infection because they had not previously received any blood or blood product.[2-4] The almost constant occurrence of post-transfusion hepatitis in patients given unheated concentrates, and concern about the transmission by blood products of the human immunodeficiency virus (HIV), have prompted manufacturers to develop procedures (usually based on various methods of heating the concentrate) for inactivating viruses with little loss of labile factor VIII activity.[5] In the past few years, several of these heated concentrates have been tested for safety in patients with hemophilia who were at risk. Although there is evidence that HIV is easily inactivated by heating and that seroconver-

From the Rehabilitation Hospital and Hemophilia Center, Rehabilitation Foundation, Heidelberg, Federal Republic of Germany; the A. Bianchi Bonomi Hemophilia and Thrombosis Center and Institute of Internal Medicine, University of Milan, Milan, Italy; the Hemophilia Center, Ospedale Policlinico, Bari, Italy; the Angiology and Coagulation Service, S. Orsola Hospital, Bologna, Italy; the Hemophilia Center, Children's Hospital, Palermo, Italy; the University Children's Hospital, Frankfurt/Main, Federal Republic of Germany; the Institute for Experimental Hematology and Blood Transfusion, Bonn, Federal Republic of Germany; Professor Hess–Children's Hospital, Bremen, Federal Republic of Germany; University Children's Hospital, Goettingen, Federal Republic of Germany; Von-Haunersches Children's Hospital of the University, Munich, Federal Republic of Germany; the Academy of Sciences, Magdeburg, German Democratic Republic; University Children's Hospital, Vienna, Austria; and the Hemophilia Center, Division of Hematology, University of Florence, Florence, Italy. Address reprint requests to Dr. Mannucci at Via Pace 9, 20122 Milan, Italy.

sion is rare in patients treated exclusively with heated concentrates,[5] all the tested concentrates were shown to transmit hepatitis.[6-10]

In 1985 we initiated a multicenter prospective study to learn whether post-transfusion hepatitis would occur in previously untreated patients with hemophilia who were given infusions of a commercial factor VIII concentrate that had been heated in solution at 60°C for 10 hours in the presence of substances (glycine and sucrose) that stabilize factor VIII activity.[11] This heating process, commonly called pasteurization, is known to eliminate the risk of transmission of hepatitis from blood products, such as albumin and plasma protein fraction, that are less labile than factor VIII. The fact that in this study no hepatitis occurred after infusion of 32 batches of the pasteurized concentrate into 26 patients indicates that pasteurization minimizes the risk of post-transfusion hepatitis.

Methods

The Concentrate

The pasteurized factor VIII concentrate (Hemate P, Behringwerke, Marburg, Federal Republic of Germany) has been commercially available since 1980. Details of the manufacturing procedure have been published elsewhere.[11] According to the manufacturers, pasteurization in the presence of stabilizers (glycine and sucrose), together with the subsequent purification step and lyophilization, results in a loss of about 30 to 40 percent of factor VIII activity. This information is in agreement with data from a study on the effects of pasteurization on factor VIII activity, indicating a loss of 30 percent.[12] Several in vivo studies[13] indicate that the biologic half-life and recovery of factor VIII activity and the effectiveness of the pasteurized concentrate in stopping bleeding in patients with hemophilia are very similar to those of unheated concentrates. To assess whether or not pasteurization inactivates the viruses responsible for post-transfusion hepatitis, manufacturers have inoculated 18 chimpanzees with unheated or pasteurized concentrates, manufactured from pooled plasma to which hepatitis B virus or plasma from patients with non-A, non-B hepatitis was added before the start of the fractionation and inactivation procedure. None of the 11 animals treated with the pasteurized concentrate contracted hepatitis, which occurred in all seven control animals treated with the same amount of the unheated concentrate.[13] In addition, in vitro studies indicate that HIV is easily inactivated under the conditions of the pasteurization process.[13]

In this study, 32 different batches of factor VIII concentrate were infused into 26 patients. Each batch was manufactured from pooled plasma collected in 1984–1985 from approximately 10,000 paid plasmapheresis donors, about 80 percent of whom were from the United States and the rest from the Federal Republic of Germany or Austria. Only plasma that was seronegative for hepatitis B surface antigen by radioimmunoassay was used in the preparation of these 32 batches. Only plasma units from donors who had normal levels of serum aminotransferases were used in the last 8 batches. The batches used in each hemophilia center were purchased from among those regularly placed on the market.

Patients

Seven hemophilia centers in the Federal Republic of Germany, five in Italy, one in the German Democratic Republic, and one in Austria enrolled 26 patients who were considered by the attending physicians to need treatment with factor VIII concentrate. Patients were selected on the basis of the criteria recommended by the International Committee on Thrombosis and Hemostasis (Miami, Fla.,

Table 1. Clinical Characteristics and Treatment of 26 Patients Given Pasteurized Factor VIII Concentrate.*

Center/ Patient	Age	Diagnosis	Factor VIII Level	Body Weight	Indication for Treatment	Total Dose of Concentrate	No. of Infusions
			%	kg		U	
01-01	22 yr	Hemophilia A	4	82	Tooth extraction	11,000	4
02-01	5 wk	Hemophilia A	10	4	Soft-tissue bleeding	750	3
03-01	1 yr, 2 mo	Hemophilia A	2	11	Soft-tissue bleeding	2,500	9
03-02	9 mo	Hemophilia A	5	8	Hernia surgery	2,000	8
03-03	1 yr, 9 mo	Hemophilia A	2	12	Soft-tissue bleeding	750	3
04-01	1 yr, 9 mo	Hemophilia A	10	13	Strabismus operation	16,500	32
05-01	2 mo	Hemophilia A	<1	4	Pylorotomy	6,500	26
05-02	3 yr	Hemophilia A	<1	14	Soft-tissue bleeding	750	6
06-01	15 yr	Hemophilia A	3	70	Soft-tissue bleeding	40,500	33
06-02	14 yr	Hemophilia A (carrier)	20	48	Tooth extraction	33,250	30
06-03	34 yr	Hemophilia A (carrier)	20	50	Gynecologic surgery	8,000	10
06-04	12 yr	Hemophilia A	9	40	Soft-tissue bleeding	5,500	1
07-01	15 yr	von Willebrand's disease	†	44	Tooth extraction	32,000	9
07-02	12 yr	von Willebrand's disease	†	30	Tooth extraction	6,000	10
07-03	5 wk	Hemophilia A	2	4	Soft-tissue bleeding	34,500	23
07-04	1 yr, 3 mo	Hemophilia A	3	10	Soft-tissue bleeding	11,500	7
08-01	17 yr	Hemophilia A	4	70	Soft-tissue bleeding	10,500	5
09-01	1 yr	Hemophilia A	<1	12	Soft-tissue bleeding	9,500	19
09-02	1 yr, 6 mo	Hemophilia A	<1	11	Epistaxis	3,000	6
09-03	1 yr, 6 mo	Hemophilia A	<1	12	Gum bleeding	2,500	5
10-01	1 yr	Hemophilia A	1	9	Soft-tissue bleeding	3,500	6
11-01	2 yr	Hemophilia A	3	12	Soft-tissue bleeding	5,000	12
12-01	22 yr	von Willebrand's disease	†	63	Postpartum bleeding	2,000	2
12-02	15 yr	von Willebrand's disease	†	58	Epistaxis	1,000	1
13-01	8 yr	Hemophilia A	1	25	Soft-tissue bleeding	1,500	3
14-01	9 yr	Hemophilia A	1	25	Soft-tissue bleeding	500	1

*The batch numbers are available from the authors.

†Factor VIII levels were normal; substitution was for prolonged bleeding times.

1984) for evaluation of the safety of clotting-factor concentrates with respect to hepatitis. Accordingly, the patients had received no previous transfusions with blood or blood products (and did not receive transfusions with blood products other than the concentrate during the follow-up period), had normal serum levels of aminotransferases, had no history or current evidence of liver disease, were not taking any medication likely to raise aminotransferase levels, had no serum marker for hepatitis B infection (except for antibody to hepatitis B surface antigen in 16 patients vaccinated against the hepatitis B virus), and had given informed consent according to the Declaration of Helsinki. These criteria were met by 20 patients with mild to severe hemophilia A, two carriers of hemophilia A, and four patients with von Willebrand's disease (the concentrate is widely used in the Federal Republic of Germany for the treatment of von Willebrand's disease because it corrects the prolonged bleeding time[14,15]).

Follow-up Protocol

Blood samples were collected and the serum was tested for aspartate and alanine aminotransferase and gamma-glutamyl transpeptidase before the first infusion of concentrate, every two weeks during the first four months, and once a month for the next two months; these blood-sampling intervals were maintained regardless of whether additional concentrate infusions were given (as recommended by the International Committee on Thrombosis and Hemostasis). Additional blood samples were collected and serum was tested for virus markers (see below) before the first infusion and then 4, 6, and 12 months afterwards. At the time of each blood sampling, the patients were seen by a physician and questioned about symptoms that might be related to hepatitis or other illnesses, and alcohol consumption and current drug therapy were recorded. Post-transfusion hepatitis was defined as the presence of alanine aminotransferase values higher than 2.5 times the upper normal limit at each laboratory on at least two consecutive occasions during the follow-up period.

Assays

Viral markers and liver enzymes were tested by the methods currently used in the central laboratories of each participating center. Markers for hepatitis B virus were tested by commercial radioimmunoassays or enzyme-linked immunosorbent assays; hepatitis A IgM antibody, by radioimmunoassay; cytomegalovirus IgM antibody and Epstein–Barr virus IgG antibody, by enzyme-linked immunosorbent assays or indirect immunofluorescence; and HIV antibody, by enzyme-linked immunosorbent assays. Liver enzymes were measured by automated and optimized colorimetric methods at 37°C.

Results

All 26 patients enrolled in the study had follow-up examinations as scheduled. Demographic data, baseline factor VIII levels, clinical indications for replacement therapy, total doses of concentrate, and numbers of infusions are listed in Table 1. Sixteen patients received infusions for the treatment of acute soft-tissue or mucosal hemorrhages; the remaining 10 were given infusions in preparation for major operations or dental extractions. Seventeen patients were given factor VIII from the same batch of concentrate throughout the follow-up period; the remaining nine patients were given factor VIII from more than one batch. In the patients with hemophilia, the clinical efficacy of the concentrate was as expected for the doses given, and the expected factor VIII levels were achieved. In the four patients with von Willebrand's disease, the prolonged bleeding time became normal after infusion. There were no immediate adverse reactions to the concentrate.

The serial levels of aminotransferases shown in Figure 1 indicate that no patient met the definition for post-transfusion hepatitis. In one patient (01-01), the serum level of alanine aminotransferase (but not aspartate aminotransferase or gamma-

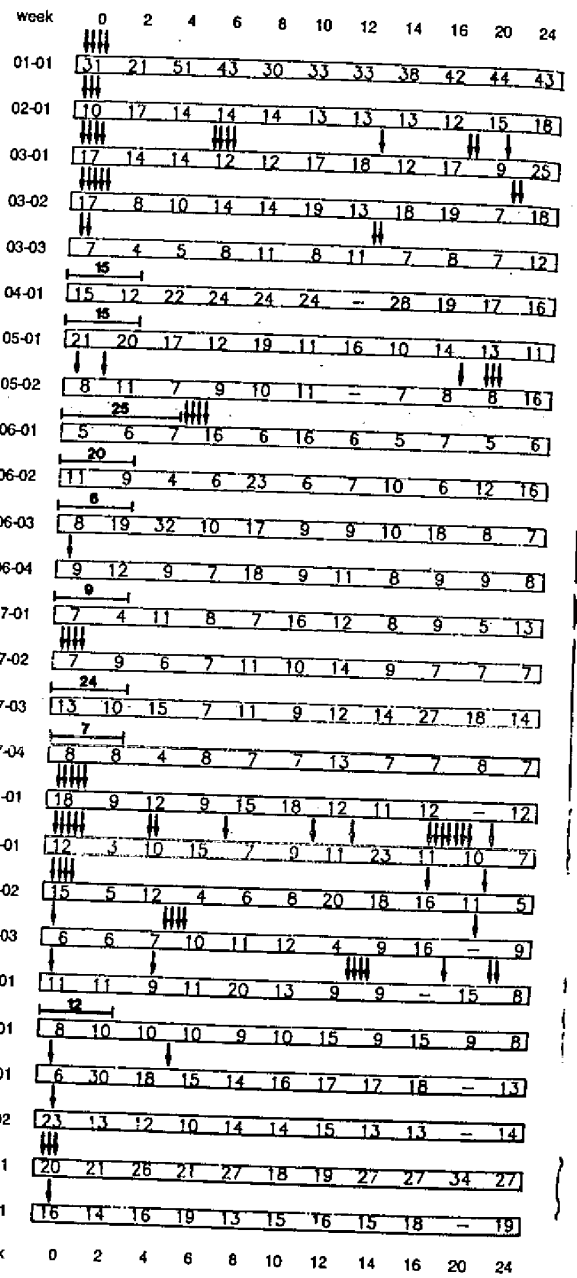

Figure 1. Serial Levels of Serum Alanine Aminotransferase (U per Liter; Upper Limit of Normal; 23 U per Liter) in 26 Patients Given Infusions of Pasteurized Factor VIII Concentrate.

The patient numbers are shown on the ordinate. Each vertical arrow indicates the time of a single infusion, and each horizontal bar indicates a period of multiple infusions; the total number of infusions is indicated above each bar. The observation period is indicated in weeks; time 0 corresponds to the first infusion of concentrate.

glutamyl transpeptidase) was slightly elevated (51 U per liter; upper normal limit, 23 U per liter) in the fourth week after infusion. In the subsequent follow-up period, the level of alanine aminotransferase remained slightly elevated (between 30 and 44 U per liter). The patient had no objective or subjective signs of disease, and he reported no alcohol intake. His base-line alanine aminotransferase level was 31 U per liter when he was admitted to the study, so that retrospectively we know that he should have been excluded.

All the patients remained seronegative for markers of infection with hepatitis B virus (except the 16 vaccinated patients who were positive for antibody to hepatitis B surface antigen from the beginning of the study), hepatitis A virus, cytomegalovirus, Epstein–Barr virus, and HIV.

Discussion

The first heated concentrate evaluated clinically with the criteria recommended by the International Committee on Thrombosis and Hemostasis — a concentrate heated at 60°C for 72 hours in the lyophilized state (commonly referred to as "dry" heating) — was highly infectious; 11 of 13 patients with hemophilia who received it acquired non-A, non-B hepatitis after transfusion.[6] Non-A, non-B hepatitis also developed in the only two patients treated with a concentrate exposed to less prolonged dry heating (60°C for 30 hours)[7] and in three patients treated with a concentrate exposed to a double virus-inactivation process (dry heating plus addition of the lipid solvent chloroform).[8] No cases of non-A, non-B hepatitis but three cases of hepatitis B occurred among 120 patients given a concentrate heated in the moistened state under steam pressure.[9] Finally, non-A, non-B hepatitis developed in 4 of 18 patients given a factor VIII concentrate heated in a suspension containing the organic solvent *n*-heptane.[10] Hence, clinical studies of heated concentrates carried out according to the protocol recommended by the International Committee indicate that dry heating is clearly ineffective in preventing hepatitis (which developed in 16 of 18 patients),[6-8] even though the severity of the clinical disease appears to be reduced.[6] With concentrates heated in the moistened state under steam pressure or in suspension (procedures commonly referred to as "wet" heating), hepatitis developed in a lower but substantial proportion of cases (7 of 38).[9,10]

The large-pool commercial concentrate used in our study was heated with a procedure (pasteurization) not previously used for clotting-factor concentrates but of established safety for other plasma fractions. No recognized case of hepatitis appeared after treatment with the heated concentrate. To have treated a concurrent control group of patients with hemophilia with the corresponding unheated concentrate from the same manufacturer would have been unethical and unfeasible, because unheated concentrates have not been licensed in our countries since it was demonstrated that HIV is highly sensitive to thermal inactivation.[13] No historical control group was available, because the incidence of post-transfusion hepatitis after use of this particular unheated concentrate had not been established when the concentrate was withdrawn from the market in 1980. Nevertheless, the similar type (paid) and origin (mostly the United States) of plasmapheresis donors, as well as the similarly large size of the plasma pool (about 10,000 donors) from which each batch was made, make it reasonable to postulate that the incidence of hepatitis associated with that concentrate should not have been much lower than that associated with other unheated concentrates (i.e., very close to 100 percent[2-4]) — in sharp contrast to the absence of hepatitis after the pasteurized concentrate.

Even so, the possibility that the pasteurized concentrate might transmit hepatitis cannot be excluded at present. The number of patients with hemophilia in this study was relatively small because of the heavy burden on such patients of a protocol involving frequent blood sampling and because of the highly selective criteria for inclusion that were used in an attempt to enroll only patients who were highly susceptible to the development of post-transfusion hepatitis. For 26 patients with no hepatitis, the one-sided 95 percent confidence intervals around the true risk of hepatitis can be calculated as 0 to 11 percent on the basis of the probability theory for binomial distribution.[16] The recent adoption of aminotransferase screening of all plasmapheresis donors and exclusion of those with high levels, as well as the exclusion of plasma donated by groups at high risk for HIV infection, should further increase the safety of the concentrate.

Even though no patient acquired antibody to HIV during this study, the follow-up period (12 months) may have been too short to allow a firm conclusion that the pasteurized concentrate is safe with respect to HIV infection. It must be emphasized, however, that the sensitivity of HIV to heating is well established[17] and that no cases of seroconversion have been seen in 18 seronegative patients with hemophilia who were treated only with this concentrate and followed for one to six years.[18]

We are indebted to Dr. Massimo Colombo for helpful criticism and advice and to Dr. Betty Rubin for reading the manuscript.

References

1. Klose HJ. Hepatitis als Schicksal des Hamophiliepatienten. In: Frosner G, Lasch H-G, Lechler E, eds. Plasmaproteine und Virushepatitis. Berlin: Springer, 1982:24-32.
2. Fletcher ML, Trowell JM, Craske J, Pavier K, Rizza CR. Non-A non-B hepatitis after transfusion of factor VIII in infrequently treated patients. Br Med J 1983; 287:1754-7.
3. Kernoff PBA, Lee CA, Karayiannis P, Thomas HC. High risk of non-A non-B hepatitis after a first exposure to volunteer or commercial clotting factor concentrates: effects of prophylactic immune serum globulin. Br J Haematol 1985; 60:469-79.
4. Morfini M, Rafanelli D, Longo G, Messori A, Rossi Ferrini P. Hepatitis-free interval after clotting factor therapy in first infused hemophiliacs. Thromb Haemost 1986; 56:268-327.
5. Gomperts ED. Procedures for the inactivation of viruses in clotting factor concentrates. Am J Hematol 1986; 23:295-305.

6. Colombo M, Mannucci PM, Carnelli V, et al. Transmission of non-A, non-B hepatitis by heat-treated factor VIII concentrate. Lancet 1985; 2:1-4.
7. Preston FE, Hay CRM, Dewar MS, Greaves M, Triger DR. Non-A, non-B hepatitis and heat-treated factor VIII concentrates. Lancet 1985; 2:213.
8. Mannucci PM, Colombo M, Rodeghiero F. Non-A, non-B hepatitis after factor VIII concentrate treated by heating and chloroform. Lancet 1985; 2:1013.
9. Morfini M, Carnelli V, Mariani G, et al. A prospective multicenter safety study of a steam heated factor VIII concentrate. La Ricerca Clin Lab 1986; 16:244. abstract.
10. Kernoff PBA, Miller EJ, Savidge GF, Machin SJ, Dewar MS, Preston FE. Wet heating for safer factor VIII concentrate? Lancet 1985; 2:721.
11. Heimburger VN, Schwinn H, Gratz P, Liben G, Kumpe G, Herchenhan B. Faktor VIII-Konzentrat, hochgereinigt und in Lösung erhitzt. Arzneimittelforschung 1981; 31:619-22.
12. Ng PK, Dobkin MB. Pasteurization of antihemophilic factor and model virus inactivation studies. Thromb Res 1985; 39:439-47.
13. Hilfenhaus J, Weidmann E. Pasteurization as an efficient method to inactivate blood borne viruses in factor VIII concentrates. Arzneimittelforschung 1986; 36:621-5.
14. Scharrer I, Vigh Z. Comparison between cryoprecipitate and a wet-heat treated factor VIII concentrate in the treatment of von Willebrand's disease. La Ricerca Clin Lab 1986; 16:231. abstract.
15. Fukui H, Yoshioka A, Nishino M, Kinoshita S, Niinomi K, Yoshioka K. Hemostatic effect of a heat-treated factor VIII concentrate (Hemate P) in von Willebrand's disease. La Ricerca Clin Lab 1986; 16:238. abstract.
16. Louis TA. Confidence intervals for a binomial parameter after observing no successes. Am Stat 1981; 35:154.
17. Spire B, Dormont D, Barré-Sinoussi F, Montagnier L, Chermann JC. Inactivation of lymphadenopathy-associated virus by heat, gamma rays, and ultraviolet light. Lancet 1985; 1:188-9.
18. Mösseler J, Schimpf K, Auerswald G, Bayer H, Schneider J, Hunsmann G. Inability of pasteurised factor VIII preparations to induce antibodies to HTLV-III after long-term treatment. Lancet 1985; 1:1111.

MEDICAL INTELLIGENCE

FAMILIAL PROPERDIN DEFICIENCY AND FATAL MENINGOCOCCEMIA

Correction of the Bactericidal Defect by Vaccination

PETER DENSEN, M.D., JOHN M. WEILER, M.D., J. MCLEOD GRIFFISS, M.D., AND LOUIS G. HOFFMANN, SC.D.

INHERITED deficiencies of various complement proteins are being recognized with increasing frequency. Careful evaluation of these deficiency states has augmented our understanding of the biologic function of individual components of the complement system, as well as of the system as a whole. Approximately half the affected persons are healthy. Nevertheless, typical clinical features have been found in the remainder, and should suggest a diagnosis of complement deficiency and identify the particular component or activation pathway that is defective.[1,2] Inherited deficiencies of the nonregulatory components of the alternative pathway have been recognized only rarely. This report describes a large family in which properdin deficiency was associated with fulminant meningococcal infection. The data emphasize the critical role of properdin and the alternative pathway in the host defense against meningococci, and provide a rationale for vaccination to prevent the potentially fatal consequences of this deficiency.

From the Department of Medicine, Veterans Administration Medical Center and the University of Iowa Hospitals and Clinics, Iowa City; the Center for Immunochemistry and the Departments of Laboratory Medicine and Medicine, University of California and Veterans Administration Medical Center, San Francisco; and the Department of Microbiology, University of Iowa, Iowa City. Address reprint requests to Dr. Densen at the Department of Medicine, University of Iowa Hospitals and Clinics, Iowa City, IA 52242.

Supported by Merit Review Awards to Drs. Densen, Griffiss, and Weiler, Clinical Investigator Awards from the Veterans Administration to Drs. Densen and Weiler, and grants (AI-16476, AI-21171, AI-22350) from the National Institutes of Health.

CASE REPORT

The proband, a previously healthy 30-year-old man who worked as a logger, presented with fulminant group Y meningococcal meningitis, from which he died. The patient's brother had died of fulminant group B meningococcal disease 16 years earlier at 18 years of age. A male cousin had had "spinal meningitis" at four years of age and "black" measles at age six, and had died of group Y meningococcal disease at age 19 during basic military training. Group Y disease was not epidemic in the recruit camp at that time.

METHODS

The buffers and cellular intermediates used in these studies were prepared as described previously.[3-8] Serum samples from individual family members were screened initially for alternative-pathway activity in hemolytic gels.[9] Subsequently, the activity of the classical and alternative pathways was quantitated by standard techniques.[10,11] Detection of properdin activity in whole serum is complicated by the presence of factors H and I, which counteract its function. Thus, properdin was quantitated immunochemically with a sandwich enzyme-linked immunosorbent assay (ELISA).[12] Zymosan and cobra-venom-factor activation of normal or properdin-deficient serum was performed as described previously.[13] At intervals, 5 μl of the reaction mixture was removed and assayed for the hemolytic activity of C3.[13] (The terminology for complement proteins that is recommended by the World Health Organization is used throughout this article.[14,15]) In some experiments, purified properdin was added to the serum (2.85 μg per 100 μl) before it was mixed with the activating agent. Antibody to meningococcal polysaccharide in serum obtained before and after vaccination was quantitated by means of a radioactive antigen-binding assay.[16] The function of this antibody was assessed in a serum bactericidal assay.[17]

RESULTS

Assay for Complement Components in Patients' Serum

Stored serum samples from the propositus, a surviving brother, and a maternal uncle demonstrated normal classical-pathway activity but an absence of alternative-pathway activity in screening assays of complement function. None of these three samples reacted with antibody to properdin in double immunodiffusion tests. The remaining complement components in the serum from the brother were normal. The addition of purified properdin to the serum