Transient Warm Autoimmune Hemolytic Anemia and Cryoglobulinemia Associated with Seminoma

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A patient with a pure seminoma presented with severe IgG-mediated warm autoimmune hemolytic anemia. Monoclonal IgM-kappa cryoglobulinemia and a biological false positive test for syphilis were also found. Treatment directed at both the seminoma and the hemolysis resulted in the complete disappearance of these antibodies. It is possible that these immunological phenomena occurred in response to the tumor. The occurrence of warm autoimmune hemolytic anemia and monoclonal paraproteinemia in association with solid tumors is reviewed.

INTRODUCTION

Warm autoimmune hemolytic anemia (WAHA) is known to occur in association with lymphoid malignancies and ovarian teratomas. No consistent association between WAHA and carcinoma has been demonstrated. Hemolytic anemia has been reported to occur in some patients with ovarian carcinoma, but in no case has the hemolysis been produced by a warm autoantibody. Although paraproteins are found in lymphoproliferative diseases, no clear association exists between the occurrence of monoclonal proteins and solid tumors. We report a patient with a pure seminoma who developed severe IgG-mediated WAHA, monoclonal IgM-kappa cryoglobulinemia and a biological false positive test for syphilis. These antibodies disappeared following treatment of the tumor and the hemolytic anemia.

CASE REPORT

In February 1973, when he was 34 years old, the patient noted enlargement of his right testicle. In March 1973 he suffered from dizzy spells and weakness and he was noted to be pale. There was a history of a congenital hydrocele of the right testis but no cryptorchidism. He had a low grade fever but no night sweats, weight loss, skin rash or Raynaud's phenomenon. There was no history of drug usage or blood loss and no prior or family history of anemia.

On examination he was pale, with a temperature of 101°F, pulse 100/min, and blood pressure 110/70 mm Hg. The skin was clear, but there was a trace of scleral icterus. There was no lymphadenopathy. A grade II/VI cardiac ejection murmur was present. The liver was palpable five centimeters below the right costal margin and the spleen tip was twelve centimeters at the level of the umbilicus. There was a small hydrocele of the left testis. A large hydrocele was present in the right testis and, in addition, a hard, irregular mass was felt fixed to the scrotum in the midline.

The hemoglobin was 5.1 gm/dl; hematocrit, 18/vol%; reticulocytes, 8/100 red blood cells.

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erythroid
gms%; albumin, 3.3

The peripheral blood smear had many microspherocytes and polychromatophilic cells and occasional nucleated red blood cells but no erythrophagocytosis. The bone marrow could not be aspirated and the biopsy was markedly hypercellular with erythroid hyperplasia and increased megakaryocytes. There was no increase in plasma cells. There was an initial suspicion that there were tumor cells present in the marrow biopsy, but there were none on the marrow “touch” preparation. In retrospect this probably represented only intense erythropoiesis.

The direct antiglobulin (Coombs') test was strongly positive (3+) with polyvalent (Ortho® and Spectra®) and monospecific anti-IgG antiglobulin. The patient's red cells were also agglutinated (2+) by antibody directed against the third and fourth components of complement (anti-C3C4). The serum and an eluate from the red cells contained an antibody that reacted with erythrocytes of all phenotypes, including 

The erythrocyte sedimentation rate was 72 mm/hr. The total protein was 6.9 gms%; albumin, 3.3 gms%; globulin, 3.6%. A discrete band in the gamma region was present on serum protein electrophoresis. Immunoelctrophoresis at 4°C revealed a precipitation line close to the origin and decreased IgA. This precipitate was identified as an IgM cryoglobulin with kappa light chains. Immunoelctrophoresis of concentrated urine revealed a small quantity of free kappa light chains. There was no cold agglutinin or rheumatoid factor. The Venereal Disease Research Laboratory test (VDRL) by slide flocculation was positive at 1:1024 dilution, but no fluorescent treponemal antibody (FTA) was present. Antinuclear and heterophile antibodies, Monospot® and lupus preparations were negative. Assays for human chorionic gonadotropin and alpha fetoprotein were not performed.

The chest x-ray was normal. Lymphangiogram revealed enlargement and architectural derangement of the lymph nodes in the abdomen. Bone density was generally increased. Liver-spleen scan revealed hepatosplenic megaly but no focal abnormalities.

Blood transfusions were given despite an inability to find compatible crossmatches, because of the severity of the anemia. The changes in clinical course and laboratory tests following therapy are depicted in Fig. 1. Prednisone was given orally, first at 100, then at 150 mg/day. 250 cc of serosanguineous fluid were removed from the hydrocele, but a 2 x 1 cm mass remained in the right testis. A right orchiectomy was performed. The tumor was a pure seminoma with a few areas of lymphoid proliferation (Fig. 2). There was no blood vessel or spermatid cold involvement. In comparison with other seminomas this lymphoid infiltrate was not marked [1]. Anemia persisted and further blood transfusions were given. One week later external radiation was delivered to the spleen, but this produced no significant reduction of its size. Because of the malignant testicular tumor, the large abdominal lymph nodes and the possibility of marrow involvement, the patient was considered to have metastatic
FIG. 1. Sequence of laboratory test results and treatment modalities utilized in the acute course of the patient: DCT—Direct Coombs' Test; ICT—Indirect Coombs' Test; Bilirubin—Total, blackened = direct; i—Red blood cell transfusion; Hct—Hematocrit; WBC—White blood cell count; CTX—Cyclophosphamide; VCR—Vincristine.
FIG. 2. H + E stain High power magnification (87x) of testicular tumor showing pure seminomatous elements and small lymphoid aggregates.

seminoma. As treatment for this, he was started on systemic chemotherapy with cyclophosphamide (1 gm/m² q 3–4 weeks) and vincristine (1.4 mg/m² weekly) two days after the end of the radiation to the spleen. After two more weeks there was a marked reticulocytosis, improvement of anemia, diminution of the direct antiglobulin score, and transient increase in the white count. The hematocrit again began to fall, and the leukopenia (3,200/mm³) and thrombocytopenia (170,000/mm³) recurred. Splenectomy was then performed.

The spleen was massively enlarged (1500 gms) and microscopically showed passive congestion and extramedullary hematopoiesis, but no tumor was found. The liver and lymph nodes were also histologically free of tumor. Immediately after the splenectomy, the white blood cell count rose to 14,000/mm³, the platelet count to 700,000/mm³ and the hematocrit to 52%. Prednisone was stopped. A repeat marrow aspirate showed only erythroid hyperplasia. Three months after the orchiectomy, the direct and indirect antiglobulin tests were negative and the VDRL and monoclonal cryoglobulin were no longer demonstrable. Hemolysis never recurred. The patient received additional chemotherapy for one and one-half years because of the uncertainty over the extent of the tumor. The lymphangiographically abnormal abdominal lymph nodes may have represented either lymphoid hyperplasia or seminoma that had already responded to the chemotherapy by the time of the laparotomy. The patient is now asymptomatic, off all treatment for two years with no recurrence of tumor, hemolysis or paraproteinemia.

DISCUSSION

Our patient developed symptomatic anemia shortly after he noted progressive testicular enlargement from a seminoma. The hemolytic anemia resulted from a warm IgG auto-antibody, which reacted as a panagglutinin, fixed complement and caused red cell destruction predominantly in the spleen. Hypersplenism, which in this
case probably resulted from a passively congested spleen, was reversed by splenectomy. The severity of the hemolytic anemia and the uncertainty over the extent of spread of the seminoma necessitated the use of several therapeutic modalities and, therefore, obscured the response to each individual maneuver. The treatments utilized that could have independently affected the course of the hemolytic anemia included orchietomy, therapeutic steroids, splenic irradiation, chemotherapy, and splenectomy. The sequence of these treatments and the hematologic responses are depicted in Fig. 1. It is conceivable that the orchietomy alone may have influenced the production of antibody and therefore hemolysis, but this is impossible to ascertain.

Although anemia in patients with malignant disease is usually related to more than one factor, hemolysis alone can sometimes be its cause. Shortened 51Cr red cell survival has been identified in patients with cancer without overt hemolytic anemia [2]. Significant hemolysis is more likely to develop as the malignancy becomes more advanced [3]. Overt hemolytic anemia, however, remains rare: clinical hemolysis was found in only 3% of anemic patients with carcinoma [2,4]. The majority of these hemolytic anemias are negative to direct antiglobulin testing (DAT) [4,5,6]. These microangiopathic hemolytic anemias are associated with mucin-producing adenocarcinomas, many involving the bone marrow [6,7,8], and demonstrate prominent red cell fragmentation, intravascular fibrin deposition, and fibrinogen and platelet consumption [9,10]. While antibody-mediated hemolytic anemia can develop in several malignancies, it is most common in the lymphoproliferative syndromes, such as chronic lymphocytic leukemia and the lymphomas [11]. These immunohemolytic anemias can be divided both clinically and serologically between IgM cold agglutinins and IgG warm agglutinins. IgM cold agglutinins fix complement and usually have specificity within the Ii erythrocyte antigen system. In contrast, IgG warm agglutinins react as panagglutinins or against weak Rh antigens, fix complement less frequently, and are demonstrable in vitro only with the addition of proteolytic enzymes or antiglobulin sera [12,12a].

WAHA occurs only rarely in association with carcinoma. In order to substantiate an etiological relationship between a carcinoma and WAHA certain criteria should be satisfied since these two diseases may occur simultaneously in the same patient by chance alone. The patient should not have a concurrent illness which itself is known to be associated with WAHA. Similarly he should not be receiving drugs known to cause a positive DAT. Serological evaluation should exclude isoantibodies and post-transfusion hemolysis, and should characterize both the eluted and serum antibody as to globulin class and specificity. A temporal relationship should exist between the WAHA and the course of the tumor. Finally, treatment of the tumor should affect the course of the WAHA. Unfortunately, very few of the reports in the literature of WAHA occurring in patients with carcinoma have satisfied all these criteria. Even if all the reported cases are accepted without critical review, their number is exceedingly small considering the occurrence of carcinoma and of WAHA. In our patient the WAHA and seminoma were closely associated temporally and the antibody was identified as an IgG warm agglutinin. Treatment of the tumor was associated with the disappearance of the antibody although this might have resulted from direct effects of therapy on the hemolytic process. One other patient has been reported with a positive DAT and seminoma but no clinical details were given [13].

Green et al [14] reported positive DAT's in 39% of 393 patients with carcinoma. This high frequency has not been supported by subsequent reports. For example, Shen et al [4] found no positive DAT's in 116 anemic cancer patients, and only one of
### TABLE 1
Summary of Malignant Ovarian Tumors Associated with Hemolytic Anemia

| Ref. # | Ovarian Tumor | Hgb/Hct (gms%/%) | Retics (%) | Spherocytes | Hemolysis | 51 Cr 1/2 life (days) | Spleen | Direct anti-globulin | Indirect anti-globulin | Auto-immune | Critique |
|--------|---------------|----------------|-----------|-------------|-----------|----------------------|-------|---------------------|----------------------|-------------|---------|
| 30 1945 | mucinous cyst-adenocarcinoma | 5.0/8.20 | + + | x ? | - | + isoantibody in Rh group | | | | | | | Hemolytic anemia improved with tumor removal. Case complicated by dermatomyositis and hemolytic transfusion reactions. Isoantibody not involved in underlying hemolytic process—no change in titer post tumor removal. |
| 31 1963 | papillary adenocarcinoma | 6.7/21.5 | 5.7 + probable | x | - | No mention | No mention | - | | | Anemia developed after tumor excision and 4500R. Uremia 2° to obstructive uropathy. Mechanism of hemolytic component unclear: ? microangiopathy. |
| 32 1966 | anaplastic carcinoma | /14 | 8.6 | R + | 8 1 cm | + (post Tx, no eluate) | + (isoantibodies K.E.c and cold aggl.) | - | | | Anemia developed after tumor excision, 1600R, and thiotepa. Coombs positive hemolysis was transient and followed blood transfusion. Mechanism: delayed hemolytic transfusion reaction. |
| 33 1969 | malignant mesenchymal epithelial carcinoma (metastatic) | 4.7/10 | + + | 5½ | - | + (IgM, eluate anti-I) | + (anti-I IgM) | - | | | Cold hemagglutinin hemolysis with hepatic sequestration. Anemia improved after removal of primary but recurred with metastatic disease. High concentration of antibody in ovarian tumor: |
| 34 1970 | cystic adenocarcinoma (metastatic) | 8/24 | 26 | + + | 3½ 2 cm | - | No mention | - | | | Anemia and hemolysis after tumor resection and 5000R. Mechanism: Hemolysin (not antibody) identified. |
| 22 1971 | malignant thecal granulosa cell | 3.8/13 | 4.2 | - | + 13 massive | - | No mention | - | | | Hypersplenism, ? myeloproliferative disease. Mechanism of hemolysis: unclear but spleen played major role. |
34 patients with cancer and hemolytic anemia reported by Hyman et al [5] was DAT positive. In an extensive review of red cell autosensitization [15], only 20 patients with carcinoma had WAHA. In four of these patients the cancer and the WAHA were clearly associated. Removal of a renal cell carcinoma in one of these patients was followed by the cessation of hemolysis and disappearance of the antibody [16]. Additional cases have been reported of WAHA occurring together with a variety of carcinomas, but no consistent pattern has emerged [3,17–20].

An association between various ovarian tumors and hemolytic anemia first described in 1938 [21] seems to be especially pertinent to our case. Unfortunately, the syndrome has been mistakenly generalized to include both benign and malignant ovarian tumors and hemolytic anemias of diverse etiology [22]. There is a clear relationship between teratomatous ovarian cystic tumors (17 of 20 reported cases) and WAHA [15,21,23–29]. In 15 cases removal of the tumor was associated with the cessation of hemolysis, and in 10 cases with the disappearance of the antibody. These reports are relevant to ours, since seminoma shares its germinal origin with the ovarian teratomas. No testicular teratomas or dermoids have been reported in association with WAHA.

The six patients reported to have simply “hemolytic anemia” in association with ovarian carcinoma [22,30–34] have followed no consistent pattern. In none of these cases was the hemolysis clearly mediated by a warm autoantibody. These reports and the mechanisms of hemolysis are summarized in Table 1 to demonstrate how they clearly differ from the syndrome of WAHA and ovarian teratoma. In two of the patients the antibodies found were isoantibodies [30,32]. One of these patients [32] was thought to have WAHA, but the course was typical of a delayed hemolytic transfusion reaction [35]. The patient reported by Andre et al [33] developed hemolytic anemia with a positive DAT. The antibody, however, was an IgM anti-I, serologically distinct from WAHA. In one patient, a potent hemolysin was identified, but it had no antibody activity [34].

In addition to the transient WAHA, monoclonal IgM-kappa cryoglobulin and a biological false positive test for syphilis were also present in our patient. There was no evidence for multiple myeloma and no symptoms (such as purpura, urticaria, or Raynaud's phenomenon) resulted from the cryoglobulin. In patients with carcinoma neither cryoglobulins [36] nor monoclonal immunoglobulins [37] occur with the increased frequency found in patients with lymphoid malignancies [36,38]. However, of those patients with “benign” monoclonal proteins, many have a carcinoma, suggesting a relationship between the two [39,40]. Treatment of a localized tumor has occasionally resulted in the disappearance of the monoclonal globulin [41]. Cryoglobulinemia can be associated with hemolytic anemia in patients with cold agglutinin disease [36] but it could not explain the WAHA in our patient.

The production of red cell autoantibody, monoclonal cryoglobulin and falsely positive test for syphilis were all transient immunological phenomena here, which may well have occurred in response to the seminoma. The stimulus for autoantibody production in a patient with a tumor is uncertain. Three mechanisms have been generally postulated for the production of red cell autoantibodies: antibodies produced to tumor-associated antigens that cross-react with red cell antigens, antibodies produced to red cell antigens altered by the tumor or antibodies produced by lymphoid elements within the tumor. However, rather than invoking such unproved local effects of the tumor, it seems equally plausible to attribute the syndrome to a systemic alteration of immunity induced by the tumor, as supported here by the presence of a variety of antibodies. This phenomenon of multiple antibody produc-
tion has also been seen in the ovarian teratoma-WAHA syndrome [11]. WAHA can occur with illnesses of very different etiology (collagen disease, lymphoproliferative disease, ovarian teratoma, alpha-methyl-DOPA administration), but the serology in all of these instances is the same. The basis of the response in the whole group may be a systemic alteration of immunity, with a resultant rather uniform pattern of autoantibody production. The exact immunological alterations caused by the tumor to account for the syndrome, such as an increase in the number of "helper" T-cells [42] or a decrease in "suppressor" cells [43], remain to be determined.

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