which was highly significant ($P < 0.0001$). We have not been able to find results compatible with this difference in the literature.

Our results for maternal blood and cord blood at delivery seem consistent with previously published values (5). The significant difference (0.28 mmol/L, $P = 0.031$) between cord arterial blood and cord vein blood lactate appears reasonable from a metabolic point of view.

The highly significant difference (0.69 mmol/L, $P = 0.009$) between maternal venous blood and cord venous blood may indicate some placental production of this metabolite. Our upper 99.7% (mean + 3s = 2.5 mmol/L) limit for CSF from normal adults seems compatible with a previously published pathological cutoff value for adults with diseases of the central nervous system (11).

Although amperometric measurements of lactate in whole blood seem to provide prompt guidance and highly acceptable laboratory support for obstetric and cardiorespiratory patients in intensive care, the instrumental design of the present lactate stat analyzer (YSI) does not completely meet our demands for an optimal stat instrument. Future developments in this area may, however, produce second-generation instruments that satisfy the stat application characteristics of easy, quick, and around-the-clock intensive laboratory service for this interesting metabolite.

We thank Alice Anderson, Herlev Hospital, for skillful assistance and cooperation in the measurements of lactate in whole blood. This work was supported by the Danish Hospital Foundation for Medical Research, Region of Copenhagen, the Faroe Islands, and Greenland, and in part by The Foundation of 1870.

References

CLIN. CHEM. 35/8 1743-1746 (1989)

Murine Monoclonal Antibody Adsorbed onto Vinlylidene Fluoride Flocules Used to Eliminate Antibody Interference in "Sandwich"-Type Immunoassays
Edward S. Newman, Laura A. Moskie, Rita N. Duggal, David M. Goldenberg, and Hans J. Hansen

We have previously reported that human anti-mouse IgG antibody (HAMA) can cause false-positive and false-negative results in "sandwich"-type monoclonal antibody (MAB) assays. To eliminate HAMA interference in "sandwich"-type MAB assays, we investigated the use of MAB on solid-phase, vinylidene fluoride flocules, which we have previously used as a solid-phase second antibody for RIA. The simple procedure effectively removes >95% of HAMA from the most positive serum we have obtained from patients hyperimmunized to murine MAB, and it allows for accurate quantification of carcinoembryonic antigen. The solid-phase complex, added to blood, effectively removes HAMA and (or) "HAMA-type" heterophilic antibody from the sera or plasma.

Additional Keyphrases: human anti-mouse antibody \* monitoring cancer \* heterophilic antibody interference

Quantification of tumor markers in blood, such as CEA, AFP, PAP, HCG, etc. is important for monitoring disease activity in cancer patients (1). Murine MABs that are reactive with these tumor-associated antigens and are labeled with various radionuclides are being widely investigated as imaging agents (2, 3). MABs to serological tumor markers are also being evaluated by many laboratories in anticancer therapy trials (2, 4). Injection of murine MABs, particularly in repeated doses, induces HAMA in many patients and HAMA can interfere with the intended activity of the MAB (5-7). HAMA induced by injection of MABs

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produces interference in "sandwich"-type immunoassays of CEA (8). Indeed, even naturally occurring heterophilic antibodies demonstrably interfere in many sandwich-type immunoassays (9-12). To monitor the concentration of CEA in serum of patients being treated with MAbs, we have used a heat-pretreatment step to destroy HAMA selectively (8, 13). However, this method is not applicable for eliminating HAMA interference in assays for other analytes such as AFP or HCG, because some analytes are heat-sensitive. Thus, we have explored alternative methods for eliminating HAMA and HAMA-type heterophilic antibodies from serum.

Here we describe the use of murine MAbs adsorbed onto porous unsintered floccules of vinylidene fluoride (VF-MAb), which can be added to samples of plasma, sera, or blood to remove HAMA or HAMA-type heterophilic antibodies. We have used our "in-house" enzyme immunoassay of CEA as a model to demonstrate the effectiveness with which this reagent eliminates HAMA interference in a sandwich-type MAb immunoassay. In developing this reagent, we used "synthetic" HAMA standards, prepared by adding primate anti-murine-MAb to CEA-negative normal human serum, as described previously (8).

Materials and Methods

MAbs: The MAbs used in this study were all of the IgG1 subclass, purified by affinity chromatography on columns of Protein A-Sepharose (14). Specificities of the MAbs we used are described below. IMMU-14 and IMMU-4 are anti-CEA MAbs; IMMU-31 is an anti-AFP MAb.

Activation of MAbs and adsorption onto VF: We activated VF (unsintered vinylidene fluoride resin powder, grade 301F; Pennwalt Corp., King of Prussia, PA) by dispersing 2.0 g of VF in 100 mL of 2-propanol and homogenizing with a Polytron (Brinkmann Instruments, Westbury, NY) for 5 min at a pulse-frequency of 4000 cycles/s. We transferred the alcohol-activated suspension of VF to a cylinder containing 1 L of PBS, stirred to disperse the suspension into the PBS, and allowed the floccules to settle. We then removed the supernate, washed the VF floccules twice with PBS by resuspending each time in 1 L of PBS, allowing them to settle, and aspirating the supernate. Finally, we added PBS to the settled pellet of VF to obtain a 20 g/L suspension of activated VF floccules. To adsorb MAb-IgG onto the VF floccules, we added to the 1-L suspension of them 100 mL of PBS containing 50 mg of MAb-IgG, homogenized with the Polytron as described above, and stirred at room temperature for 3-4 h, then at 4 °C for at least 12 h. We removed unadsorbed MAb-IgG by low-speed centrifugation for 10 min, then discarded the supernate, reconstituted the pellet to the original volume of PBS, and washed the pellet twice more with PBS. We then suspended the pellet in 1 L of PBS containing 100 mg of thimerosal (preservative) and 1.5 g of human serum albumin and homogenized with the Polytron as described above. This suspension of VF-MAb was stored at 4 °C until used to adsorb HAMA or heterophile antibody from serum.

HAMA adsorption with VF-MAb: We pipetted 5.0 mL of VF-MAb into 13 × 100 mm glass test tubes and centrifuged (1.5 × g, 10 min, room temperature). The supernate was decanted and discarded and the tubes were drained. We added 1.0 mL of serum, plasma, or blood to the VF-MAb pellet in the different test tubes and vortex-mixed to resuspend the pellet. The suspension was incubated at room temperature for 10 to 15 min and centrifuged as before, and the supernate was decanted and saved for assay of HAMA and CEA.

Enzyme immunoassays: To quantify CEA in sera and in heat-extracted sera, we used a research sandwich-type enzyme immunoassay (EIA), "ImmuSTRIP CEA/MA-EIA," developed by Immunomedics, Inc. (8). We determined the titer of HAMA of each specimen by using a commercially available research assay, "ImmuSTRIP HAMA-EIA" (Immunomedics, Inc.), a direct assay in which polyclonal-IgG is bound to the solid phase and the probe is polyclonal-IgG conjugated to horseradish peroxidase (EC 1.11.1.7); antimurine-IgG antibody of any subclass or species that will bridge the probe to the solid phase can be accurately quantified.

Primate anti-murine-monoclonal antibody: We hyperimmunized baboons with IMMU-4-IgG (15). The resulting antisera were assayed for anti-murine IgG activity with the Immunomedics ImmuSTRIP HAMA-EIA.

"Synthetic" anti-murine-MAb controls: To obtain "synthetic" anti-murine-MAb controls, we diluted primate anti-murine-MAb-sera into a pooled specimen of human serum that had been proven to be free of CEA when assayed by commercial CEA-EIAs (8).

Results

With the protocol we used to adsorb HAMA and "HAMA"-type heterophilic antibody from blood or serum we could adsorb >95% of "HAMA-like" activity of normal human serum to which baboon anti-IMMU-4 serum had been added. Elsewhere, we have discussed the use of this reagent to evaluate HAMA-interference in immunoassays (8). All three VF-MAb preparations, involving MAbs of different specificities, were equally effective in removing HAMA from the control serum. Indeed, we have observed that VF-MAb made with immunizing murine MAb, IMMU-4, is no more effective in removing HAMA than is VF-MAb made with IMMU-31, a MAb reactive with AFP, or IMMU-14, a MAb reactive with CEA. The data also demonstrate that the false-positive interference produced by HAMA in our CEA-EIA was completely eliminated by pre-adsorption of the control with all three VF-MAb reagents; the titer of the CEA-negative control was >50 μg/L before adsorption and <2.5 μg/L after adsorption.

To demonstrate that VF-MAb will effectively remove HAMA induced by injection of murine-MAb (IMMU-4) from the sera of cancer patients, we adsorbed the sera of the six patients with VF-MAb prepared by using the anti-AFP-MAb, IMMU-31. Table 1 shows the results. HAMA was effectively removed from the sera, and HAMA inter-

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<th>Table 1. Elimination of HAMA Interference in CEA Analysis by Adsorption (ad) of Six Sera with VF-MAb-IgG (IMMU-31)</th>
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<td><strong>HAMA, mg/L</strong></td>
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<td>Pre-ad</td>
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<td>123.8</td>
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* CEA titer determined with heat-extraction assay described in ref. 8. n.d., not determined.
ference of the CEA-EIA was minimized. Data shown in Table 1 for patients 4, 5, and 6 demonstrate that adsorption of sera with the VF-MAB-IMMU-31 does not remove CEA from the sera. The CEA concentration determined when heat is used to destroy HAMA is essentially identical to that determined after adsorption of HAMA with VF-MAB.

To demonstrate that HAMA could be adsorbed from whole blood, we drew blood into tubes containing EDTA and into tubes containing no anticoagulant, and immediately supplemented the blood with HAMA (50 µL of primate anti-IMMU-4 in about 3 mL of blood). One milliliter of this mixture was promptly pipetted into tubes containing pellets of VF-MAB-IMMU-31. The tubes containing EDTA-treated blood were immediately centrifuged, and the plasma was transferred to another tube. We allowed the blood in other tubes to clot, centrifuged the tubes, and transferred the serum into another set of tubes. In addition to the blood supplemented with HAMA, we obtained blood from a normal volunteer known to have heterophilic antibody reactive with murine-IgG and treated it like we treated the HAMA-supplemented blood. Typical results were obtained upon assay for HAMA (8.0 mg/L decreased to 0.08 mg/L after the adsorption) and the natural heterophilic antibody (0.29 mg/L decreased to 0.06 mg/L after adsorption), demonstrating the effective removal of HAMA from the blood (Table 2). We have titered the amount of VF-MAB required to remove 0.1 to 1.0 mg of HAMA per liter from blood. Our preliminary data indicate that as little as 50 µL of a 20 g/L suspension of VF-MAB will remove 1.0 mg of HAMA per liter from blood.

Discussion

Our results clearly demonstrate that VF-MAB will adsorb HAMA and naturally-occurring HAMA-like heterophilic antibodies from serum and blood. Our preliminary experiments demonstrate the feasibility of preventing interference by HAMA in a MAB sandwich-type assay for AFP. VF-second antibody has been used in immunoassays for many analytes—including CEA, AFP, HCG, estradiol—and enzymes such as CK-MB and PAP (16). Thus, VF-MAB probably will have broad application for eliminating HAMA from serum or blood without adsorbing the analyte itself. VF-MAB also has the potential to eliminate HAMA-type heterophilic antibody interference in sandwich-type immunoassays, a problem recently described by other investigators (9–12). Indeed, the reagent could be added to evacuated blood-collection tubes during manufacture of the devices.

Although HAMA interference of sandwich-type murine-MAB assays can be minimized by adding polyclonal or monoclonal IgG to the reagents, the immune complexes might block antigen binding on sites on the solid phase, resulting in false-negative results (8). Furthermore, it has not been possible in other cases to eliminate false-positive interference completely when the serum contains high concentrations of HAMA (17). This could be due to incomplete adsorption of HAMA or bridging of the probe to the solid phase by immune complexes. Additionally, studies have not yet demonstrated that the immune complexes do not produce a matrix effect, an effect that may be most likely to occur in sera that have increased concentrations of immunoglobulins and acute-phase proteins. Adsorption of HAMA onto VF-MAB effectively removes HAMA from the specimen.

Additional studies are in progress to define the composition required to produce a universal VF-MAB, because a blend of MABS of different subclass and different isotypes may adsorb HAMA induced by different MABS. The MABS used in the CEA-EIA that we utilized in this study were of the IgG1 subclass, as were also the MABS used to manufacture the VF-MAB preparations. All MABS used also had the same light-chain isotypes. Patients injected with a MAB of IgG3, subclass may have a significant HAMA that is not reactive with VF-MAB-IgG1. Predictably, the HAMA left in the serum after adsorption with VF-MAB-IgG1, which is specific for IgG3, would not interfere in an in vitro assay in which IgG1 MABS are used. However, if the assay involves one or two MABS of IgG2a, the HAMA left in the sera could interfere.

In summary, treatment of serum or blood with VF-MAB appears to be an effective and simple way to remove HAMA or natural MAB-reactive heterophilic antibody that can interfere in murine-MAB in vitro immunoassays. However, use of VF-MAB is not recommended unless the reagent is demonstrated not to adsorb the analyte and to prevent false-positives caused by HAMA. The use of primate anti-MAB sera to create controls with known amounts of HAMA and analyte provides authenticated reference material for validating the effectiveness of this reagent.

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References

Neopterin as a Predictive Marker for Disease Progression in Human Immunodeficiency Virus Type 1 Infection

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We assessed the value of urinary neopterin concentrations for prognosis of disease progression in HIV-1-infected patients. Sixty-eight anti-HIV-1 seropositive homosexuals with lymphadenopathy syndrome were tested for urinary neopterin and T-cell subset counts in 1982–83, and the incidence rate at which they developed acquired immunodeficiency syndrome (AIDS) between then and May 1988 was evaluated. Overall, 21 of 68 (30.9%) cases progressed to AIDS, with a yearly progression rate of 4–9%. The predictive value of urinary neopterin concentrations was higher (P = 0.0042) than that of CD4+ T-cell counts (P = 0.015) or the CD4+/CD8+ T-cell ratio (P = 0.022). Counts of CD8+ T-cells failed to show predictive significance (P = 0.29). Similarly, multivariate-regression analysis indicated that neopterin concentrations and CD4+/CD8+ T-cell numbers were significant copredictors. Produced by human macrophages activated by interferon gamma, neopterin is thus a marker of macrophage activation via T cells. We conclude that these data demonstrate a correlation between the amount of T-cell-macrophage activation, as measured by urinary neopterin concentrations, and the progression of the disease.

Human immunodeficiency virus type 1 (HIV-1) is the causative agent for the acquired immunodeficiency syndrome (AIDS), which was first reported in the United States in 1981 (1).

Several reports have addressed the problem of early prediction of HIV-1-related disease progression. Low numbers of CD4+ T-helper/inducer cells and low ratios of CD4+ T cells per CD8+ cytotoxic/suppressor T cells were shown to be associated with more unfavorable disease course (2–9). Additionally, HIV-1 p24 antigenemia (8), increased concentrations of β2-microglobulin in serum (8), and increased urinary concentrations of neopterin (10) indicated more rapid progression of the disease.

In 1982–83, we measured urinary neopterin concentrations in a group of patients with generalized lymphadenopathy. Early results of that study have been published elsewhere (II, 12). Now we have analyzed the association between urinary neopterin concentrations and the likelihood of progression towards AIDS in the period since the initial neopterin determinations. For comparison, we performed analogous analyses for quantifying CD4+ and CD8+ T cells and determining the CD4+/CD8+ T-cell ratios, concomitantly with measurements of neopterin.

Materials and Methods

Patients: The study population of 68 patients was enrolled in 1982 and the first half of 1983. These patients are a randomly chosen subset of an entire cohort described in detail earlier (2, 9). All had HIV-1-associated lymphadenopathy syndrome and some had various constitutional symptoms. All were homosexual or bisexual men and were shown retrospectively to have been anti-HIV-1 seropositive already at their initial visit by ELISA (Abbott Laboratories, North Chicago, IL) confirmed by Western blot.4

Assay methods: Neopterin was determined in first morning urine specimens by a reversed-phase "high-performance" liquid chromatography (HPLC) method described elsewhere (13), which allows simultaneous determination of urinary creatinine. Neopterin concentrations were related to creatinine values to compensate for physiological variations in the analyte concentrations in urine.

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4 Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Dept. of Health and Human Services.