lysts 13 times over a 1-month period was 6.0% (mean net bilirubin absorbance, 0.0346 ± 0.0021). The intraday CV was 3.3% on this sample (mean absorbance, 0.0377 ± 0.00123, n = 12).

The correlation between serum bilirubin and CSF bilirubin was −0.058 (n = 31), indicating that the CSF bilirubin absorbance was not because of diffusion of bilirubin into the CSF or a consistent contamination of the CSF with vascular blood during the LP. In all our positive results to date, the clinical follow up has confirmed positive xanthochromia.

In conclusion, this test is simple, inexpensive, can easily be interpreted, and thereby overcomes the confusion in diagnosing subarachnoid hemorrhage (6–8). We have used it for the last 20 months in over 50 patients, and it has proven clinically useful.

References

Interferences with Two-site Immunoassays by Human Anti-mouse Antibodies Formed by Patients Treated with Monoclonal Antibodies: Comparison of Different Blocking Reagents, Jochen Reinsberg (Zentrum für Frauenheilkunde und Geburtshilfe, Universität Bonn, Sigmund-Freud-Strasse 25, D-53127 Bonn, Germany; fax 49-228-2874651)

The most widely used approach for reducing interferences with two-site immunoassays by human anti-mouse antibodies (HAMAs) developed by many patients after exposure to murine immunoglobulins is to include high amounts of nonspecific mouse IgG within the assay buffer (1, 2). Recently, Mössner et al. (3) have developed a polymerized form of murine IgG (MAK33), which they found to be superior to normal mouse IgG for blocking HAMA interferences. In contrast, we observed false-positive values for the cancer antigen 125 (CA 125), because of HAMA interferences in samples from ovarian cancer patients treated with the anti-CA-125 antibody OC125, which could be corrected by preincubation with mouse IgG but not with MAK33 (4). The aim of the present study was to examine whether treatment with other monoclonal antibodies gives rise to additional HAMAs that are insensitive to MAK33 and to further characterize this specific HAMA response.

Sixty-four serum samples were obtained from 51 ovarian cancer patients who, in the course of several clinical studies (5–8), had received multiple infusions of one of four murine monoclonal antibodies: OC125 (16 patients), B43.13 (8 patients), ACA125 (18 patients), and B72.3 (9 patients). The procedures followed in this study were in accordance with the standards of the ethical committee of our faculty. The interfering HAMA activity of the samples was quantified with a bridging HAMA assay (HAMA-ELISA medac, Medac) involving polyclonal murine IgG in both capture and detection steps before and after preincubation (30 min at room temperature) with the following: (a) polyMAK-33 (MAK33), a polymerized murine IgG₃ (γ) preparation (gift of Boehringer Mannheim, Mannheim, Germany); (b) Immunoglobulin Inhibiting Reagent (IIR) a formulation of immunoglobulins targeted against HAMAs (gift of Bioreclamation, East Meadow, NY); (c) polyclonal mouse IgG (reagent grade, Sigma Chemical Co.); and (d) mouse IgG of different subclasses, purified from myeloma proteins (Sigma) of the plasmacytoma cell lines UPC-10, HOPC-1, MOPC-141, and FLOPC-21.

Comparison of blocking reagents. In 35 samples, the interfering HAMA activity (ranging from 1162 to 55 397 µg/L) could be blocked completely by each of the three blocking agents (HAMA activity <100 µg/L after preincubation with 0.7 g/L MAK33, 2 g/L IIR, or 0.7 g/L polyclonal mouse IgG). In contrast, in 29 samples, the interfering HAMA activity (ranging from 1160 to 1 648 000 µg/L) could be eliminated only by IIR and polyclonal mouse IgG, respectively, whereas after preincubation with MAK33, increased HAMA values were still measured (Fig. 1). Residual HAMA activity insensitive to MAK33 was observed in most samples obtained from patients treated with OC125 fragments (16 of 18) compared with a considerably lower incidence in the other treatment groups (5 of 16 treated with B43.13, 4 of 19 treated with ACA125, and 4 of 11 treated with B72.3).

Specificity of the HAMA response insensitive to MAK33. To characterize the specificity of the HAMAs insensitive to MAK33 and to clarify how far they are directed against isotypic determinants not expressed on the gamma, heavy or the kappa light chain presented by MAK33, six samples (three OC125 patients, two ACA125 patients, and one B72.3 patient) were preincubated with supplementary 0.3 g/L murine IgG of different subclasses in the presence of 0.7 g/L MAK33. Nearly complete inhibition (73–99%) was achieved in all six samples by the IgG₂a₃, antibody UPC-10, whereas the IgG₂a抗体 HOPC-1 and the IgG₃ antibody FLOPC-21 were ineffective in two samples, and in two samples only weak inhibition (17–35%) was observed. This indicates that the HAMAs of at least four samples bind to epitopes on the UPC-10 antibody.
that are not related to the isotype of the gamma2a heavy or kappa light chain but rather are allo- or idiotypic epitopes. When we compared the specificity of the HAMAs formed by the different treatment groups, we found similar effects of the antibodies MOPC-141 (IgG2b, kappa) and FLOPC-21 (IgG3, kappa) on samples of the same treatment group, but marked differences between the treatment groups: There was no inhibition (10%) of the ACA125-induced HAMAs by the MOPC-141 antibody compared with quite high effects on the OC125 (43%, 49%, and 76%) and B72.3-induced HAMAs (66%); the FLOPC-21 antibody had no effect on the B72.3- and ACA125-induced HAMAs (>10%) compared with a weak inhibition of the OC125-induced HAMAs (17%, 35%, and 64%). However, for the two samples obtained from ACA125-treated patients, we found markedly different inhibitions of 80% and >10% by the HOPC-1 antibody, indicating that the specificity of the HAMA response may be variable even within the same treatment group.

Effect of multiple antibody infusions. As shown in Table 1, in serial samples drawn from two patients treated with the antibodies OC125 and B72.3, respectively, the percentage of the HAMA activity remaining after preincubation with MAK33 markedly increased with the number of antibody infusions.

The present results demonstrate that, in patients treated not only with the murine antibody OC125 but also with the antibodies B43.13, ACA125, or B72.3, HAMAs are formed that are insensitive to MAK33. Because MAK33 is prepared from a monoclonal antibody of the IgG1 subtype (3), and the antibodies OC125, B43.13, B72.3, and ACA125 also belong to the IgG1 subtype (9–12), we must suppose that all HAMAs formed against the isotypic determinants of these four antibodies will be blocked by MAK33. If this is true, we also would assume that the HAMAs insensitive to MAK33 are directed against allotypic or idiotypic epitopes (idiotopes) not related to the isotype of the IgG1 antibodies. This assumption is further confirmed by the results of the blocking experiments with the different murine IgG subclasses.

Although allotypic epitopes can be expressed by many IgG molecules independent of their isotype, idiotopes that are formed by the variable region on the Fab portion mostly are unique epitopes. However, within the framework of the variable region there exist so called public or cross-reactive idiotopes that are expressed on several antibody clones (13). Thus, we can assume that interferences by specific HAMAs, which are directed against certain allotypic epitopes or cross-reactive idiotopes expressed on both capture and detector antibodies of an immunoassay but not on the MAK33 antibody, cannot be corrected by addition of MAK33. However, we can expect that the respective epitopes are expressed at least on a small fraction of polyclonal murine IgG prepared from many animals, which will be suitable to mask the corresponding HAMA activity.

Our data show that the percentage of the HAMA activity insensitive to MAK33 markedly increased with the number of antibody infusions. Thus, we presume that the specific HAMA response insensitive to MAK33 only becomes apparent in patients treated repeatedly with the same monoclonal antibody. This is in accordance with other reports that describe an early HAMA response to the isotypic epitopes followed by an increasing specificity.

### Table 1. Variation of HAMA response with number of antibody infusions. a

<table>
<thead>
<tr>
<th>Patient</th>
<th>Antibody</th>
<th>Number of infusions</th>
<th>HAMA activity, µg/L Total</th>
<th>HAMA activity, µg/L After MAK33</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>G.B.</td>
<td>B72.3</td>
<td>4</td>
<td>6733</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>1536</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
<td>7486</td>
<td>900</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>1555</td>
<td>230</td>
<td>15</td>
</tr>
<tr>
<td>M.G.</td>
<td>OC125</td>
<td>2</td>
<td>1571</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1805</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>8670</td>
<td>1850</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>31 700</td>
<td>9930</td>
<td>31</td>
</tr>
</tbody>
</table>

a HAMA activity was measured before and after preincubation with MAK33 in serial serum samples drawn from two patients during therapy with multiple infusions of the antibodies OC125 and B72.3, respectively.
for the idiotypic epitopes after repeated infusion of murine antibodies (14, 15). Furthermore, this could explain why Mössner et al. (4) found a better efficacy of MAK33 compared with polyclonal murine IgG, because they studied the effect on HAMA-like heterophilic antibodies in serum samples from healthy subjects who had been immunized by a diversity of unknown immunogens. However, how attributable the high incidence of the specific HAMA response in OC125-treated patients is to infusion of F(ab’)2 fragments must be clarified by additional studies.

As shown by the blocking experiments with the different murine IgG subclasses, the specificity of the HAMA response insensitive to MAK33 obviously is dependent on the specificity of the antibody used for immunization, but it can also vary within the same treatment group. As confirmed by similar observations of Vaidya and Beatty (16), polyclonal murine IgG prepared from many animals seems to be the best reagent to block interferences by such HAMAs because it will present all allotypic and cross-reactive idiotypic epitopes expressed on murine IgG molecules, whereas blocking reagents based on a single monoclonal antibody, such as MAK33, can be unsuitable when they are missing the respective epitopes. The HAMA-blocking reagent IIR also appears to be an effective reagent for eliminating interferences by HAMAs directed against allotypic or cross-reactive idiotypic epitopes. The IIR is a mixture of murine monoclonal antibodies produced by immunization with human heterophilic antibodies and HAMAs; antibodies specifically directed against the HAMA protein were screened and selected using the same material. These antibodies appear to bind all the active sites on the HAMA moiety with high affinity, thus preventing its binding to the reagent antibodies of a murine-based immunoassay. From the present data, we cannot decide whether this “active” principle of HAMA blocking is responsible for the good efficacy of the IIR or whether the reagent is a mixture of several murine antibodies that collectively express all epitopes needed to block HAMA interferences.

In summary, our data demonstrate that, in patients treated repeatedly with the same monoclonal antibody, a considerable percentage of the HAMA response is directed against allotypic and cross-reactive idiotypic determinants. Interferences by these HAMAs can be blocked by polyclonal murine IgG; however, reagents like MAK33, which are designed for the blocking of heterophilic antibodies found in healthy subjects, may be ineffective. Because of the high variability of the specificity of the HAMA response, it seems to be difficult to find one monoclonal antibody that is effective as an universal blocking reagent. However, an alternative to polyclonal murine IgG may be a reagent made up by several different purified monoclonal antibodies. Such a reagent has a constant composition and should give a reproducible performance.

I thank Boehringer Mannheim and Bioreclamation Inc. for providing the HAMA-blocking reagents MAK-33 and IIR and D. Ackermann and E. Jost for skilled technical assistance.

References


Eleysys Testosterone Assay Evaluated, Marta Sánchez-Carbayo, Montserrat Mauri, Rocio Alfayate, Carmen Miralles, and Federico Soria (Laboratorio de Hormonas, Servicio de Análisis Clinicos, Hospital General Universitario de Alicante, C/ Maestro Alonso 109, 03010 Alicante, Spain; * author for correspondence: fax 34-6-590-8524)

Serum testosterone (T) is one of the most commonly requested human steroids. In women, measurement of serum T concentration is useful in evaluating hirsutism, alopecia, and menstrual disorders. In boys and men, its utility is related to the investigation of testicular dysfunction. It has also been used to monitor the treatment of patients with congenital adrenal hyperplasia, as well as the antiandrogen therapy of patients with prostate cancer (1–3).