congenital adrenal hyperplasia (steroid 21-
hydroxylase deficiency). J Inherited Metab

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Circulating Antibodies to Mouse
Monoclonal Immunoglobulins
Caused False-Positive Results in a
Two-Site Assay for
Alpha-Fetoprotein

To the Editor:

Previously we reported on a patient
who underwent an adjuvant chemother-
yapy because of spurious increas-
end-alpha-fetoprotein (AFP) val-
ues (1). Comparison of different test
systems revealed that the values in a
one-step homologous mononuclear
assay were falsely increased, whereas
with polyclonal assays all results were
negative. The high AFP concentra-
tions of the monoclonal assay could
not be abolished by including nonim-
mune serum. The factor that was
responsible for the false increase could
be purified and was identified as an
IgG molecule (2). The results impli-
cate the existence of a heterophilic
antibody, forming a bridge between
the two mouse antibodies in the test
system. This phenomenon is suf-
fi ciently known and can result in errors
in analyte quantification in any tech-
nique involving antigen binding to re-
agent antibody (for a review, see 3).
Usually these interferences are poten-
tially serious pitfalls and lead to a
great deal of unnecessary follow-up
testing, including invasive proce-
dures. A remarkable fact in the pre-
cent case is that the patient was not
treated with mouse antibodies, nei-
ther for diagnostic nor for therapeutic
purposes, and that at his work he has
no contact with animals whatsoever.

That is why the etiology for the forma-
tion of the heterophilic antibody re-
mained unsolved so far.

To obtain clues pertaining to the
putative mechanism of its origin, we
analyzed all drug preparations that
the patient had taken other than those
used in the chemotherapy. Altogether,
he had taken 18 different prepara-
tions, exclusively immune-stimu-
lating substances of partly vegetable,
partly animal origin. First, using so-
dium dodecyl sulfate gel electropho-
resis, we analyzed all preparations to see
whether they contained proteins of a
high molecular mass. We found that
the preparations Apia prophyca
(Laboratorium Apia, Lahr, F.R.G.),
Wobe-Mugos, and Wobenzym (both
from MUCOS Pharma, Geretsried,
F.R.G.) contain polypeptides with a
molecular mass >10 000 Da. Espe-
ically these preparations, but also all
others, were tested to determine
whether they would react with the
patient's IgG fraction. For that we
used a Western blot analysis: the sam-
ple were transferred electrophoret-
ically onto nitrocellulose filters after
electrophoretic separation, and strips
with the preparations were incubated
with the patient's serum. For sub-
sequent visualization we stained with a
rabbit anti-human IgG-horseradish
peroxidase conjugate. Only the
Wobenzym and not the other prepara-
tions showed a positive reaction.

To re-establish the link to the AFP
kit and that to the mouse antibodies,
we carried out competitive inhibition
experiments with all preparations
containing high-molecular-mass pro-
tein. Cross reaction of the heterophilic
antibody between a preparation and
the mouse IgG should remove the false
positivity in the monoclonal AFP kit.
The patient's serum was mixed with
all preparations in different dilutions,
all adjusted to the same protein con-
centration. After pre-incubation, we
monitored the titer of the mixtures by
measuring the inhibition effect in the
monoclonal AFP kit. The undiluted
Wobenzym solution almost completely
inhibited the AFP. Increasing dilution
diminished the inhibition effect. Apia
prophyca had a minor effect, which
was exceeded by Wobenzym in a mag-
itude of five log-steps. The inhibition
effects of all other preparations were
negligible.

We conclude that the patient was
unintentionally immunized with the
preparation Wobenzym. The repeated
antigen challenge provoked hetero-
philic antibodies cross-reacting with
mouse IgG. This conclusion is sup-
ported by the time of taking the prepa-
rations and the following course of
AFP values, which reverted to normal
without any further therapy (Figure
1). In case of uncertain test results,
especially in mouse monoclonal "sand-
wich" systems, doctors should pay at-
tention to whether patients have
taken preparations of animal origin.

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