We read with interest the recent paper of Uchida et al in which they indicate that GM-CSF AAs are complexed with residual antigen are used. In our hands, an electrochemiluminescence-based assay tolerant for up to 5 ng/mL GM-CSF is often included in antibody detection protocols. Alternatively, novel electrochemiluminescence-based assays which detect antibodies even in the presence of non–GM-CSF proteins (ie, non–GM-CSF). Further confirmation of specificity can be obtained by preincubating the antibody-positive samples with an excess of GM-CSF before inclusion in the assay. A reduction in signal with GM-CSF treated samples as opposed to untreated antibody-positive samples would confirm that the binding is due to the presence of genuine anti–GM-CSF AAs. While the authors have shown that sera containing anti–GM-CSF AAs bind to both yeast- and Escherichia coli–derived recombinant GM-CSF, in our experience using E coli–derived GM-CSF, detection of anti–GM-CSF AAs in sera from cancer patients and healthy persons is very rare. However, binding to yeast-derived recombinant GM-CSF and other yeast-expressed proteins (non–GM-CSF) may be problematic due to the presence of antibodies to yeast glycans. The authors indicate that GM-CSF AAs are complexed with circulating GM-CSF and are present as immune complexes, but the approaches used for this appear to lack validation. For detecting antibodies that are complexed with the antigen, an acid dissociation step is often included in antibody detection protocols. Alternatively, novel electrochemiluminescence-based assays which detect antibodies even in the presence of residual antigen are used. In our hands, an electrochemiluminescence-based assay tolerant for up to 5 μg/mL GM-CSF detected antibodies against GM-CSF in 8% of sera from healthy controls. Inclusion of an acid dissociation step did not increase the number of anti–GM-CSF AA–positive sera. These antibodies did not neutralize GM-CSF–induced proliferation of TF-1 cells and were therefore nonneutralizing.

To the editor:

Are neutralizing anti–GM-CSF autoantibodies present in all healthy persons?

We read with interest the recent paper of Uchida et al1 in which they show that neutralizing anti–granulocyte-macrophage colony-stimulating factor (GM-CSF) autoantibodies (AAs) occur in all healthy persons. Previously, high-titer neutralizing anti–GM-CSF AAs have been reported by Uchida et al (2004)2 in sera from most patients with idiopathic pulmonary alveolar proteinosis (PAP) and by us in rare patients with myasthenia gravis,3 but not in sera from healthy controls (HCs). Indeed, even non-neutralizing GM-CSF AA are relatively rare in sera from healthy controls4,5 or autoimmune disease6,7 Nevertheless, low-titer neutralizing anti–GM-CSF AAs have been found in human intravenous immune pulmonary alveolar proteinosis patients in Japan. The authors indicate that GM-CSF AAs are complexed with circulating GM-CSF and are present as immune complexes, but the approaches used for this appear to lack validation. For detecting antibodies that are complexed with the antigen, an acid dissociation step is often included in antibody detection protocols. Alternatively, novel electrochemiluminescence-based assays which detect antibodies even in the presence of residual antigen are used. In our hands, an electrochemiluminescence-based assay tolerant for up to 5 μg/mL GM-CSF detected antibodies against GM-CSF in 8% of sera from healthy controls. Inclusion of an acid dissociation step did not increase the number of anti–GM-CSF AA–positive sera. These antibodies did not neutralize GM-CSF–induced proliferation of TF-1 cells and were therefore nonneutralizing.

References


The authors have provided only scant information regarding the performance of the GM-CSF affinity column used to isolate antibodies. If used repeatedly, is it possible that a proportion of high affinity anti-GM-CSF AAs from PAP sera used as a positive control would be retained on the GM-CSF column and released slowly during subsequent purifications of HC IgG? If PAP anti-GM-CSF IgG contamination did occur, not only would it account for the immunoblot data given in Figure 1a in their paper,1 where radiolabeled GM-CSF was shown to bind to the bound (IgG) fraction from both HC and PAP IgG, but also for the positive results in bioassays. Evidence of absolute clearance of GM-CSF-bound proteins before column reuse would have been useful in eliminating this possibility.

Finally, the functional assays used for GM-CSF, including those used for measuring neutralizing effects, do not appear to have been adequately controlled for specificity. Assays used for measuring GM-CSF activity are not specific as they can respond to a range of cytokines and can be affected by inhibitory components in sera. Therefore, if specificity for neutralizing GM-CSF is to be claimed, then it is necessary to show that the affinity-purified antibodies do not neutralize other cytokines, such as interleukin-3, which can be tested using the TF1 cell-proliferation assay.13

To the editor:

Acid sphingomyelinase deficiency does not protect from graft-versus-host disease in transplant recipients with Niemann-Pick disease

Rotolo et al reported1 that ceramide, generated from sphingomyelin by acid sphingomyelinase (ASM) and coalesced into plasma membrane platforms, is necessary for transmembrane relay of cytotoxic T-lymphocyte (CTL) signals, such as Fas-FasL signals, critical in the effector stage of acute graft-versus-host disease (GVHD). Due to defective CTL-mediated lysis of ASM-deficient organs, recipients had reduced GVHD lethality and target organ injury, especially of the gastrointestinal tract and liver. In addition, GVHD amelioration was also associated with increased donor T-cell apoptosis and reduced proinflammatory cytokine responses.

To determine whether the effects of ASM deficiency in mice were translatable to humans, we examined the incidence of acute and chronic GVHD in patients with ASM deficiency, termed Niemann-Pick (NP) disease. Approval was obtained from the University of Minnesota’s Institutional Review Board for these studies. Informed consent was provided according to the Declaration of Helsinki. At least 4 types of NP disease exist, 2 of which have either complete (neurovisceral form, NP type A, or NPA) or partial (visceral form, NP type B, or NPB) constitutional deficiency of ASM.2 In both NPA and NPB, ASM substrate (sphingomyelin) accumulates to the same degree in visceria, and leads to progressive multiorgan dysfunction and death. Because phenotypic cross-correction is feasible in ASM-deficient mice,3 hematopoietic cell transplantation (HCT) has been proposed as a therapy for both forms.

References


Conflict-of-interest disclosure: The authors declare no competing financial interests.
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