

FOUR PATIENTS IN SINGAPORE WITH ANTI-GOLGI ANTIBODIES

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ABSTRACT

Serum specimens for anti-nuclear fluorescence tests are routinely received in our laboratory. Four specimens were spotted to be negative for anti-nuclear fluorescence but positive for fluorescence characteristic of that caused by anti-golgi antibodies.

(a) Patient A had acute glomerulonephritis;

(b) Patient B had acute viral hepatitis;

(c) Patient C had deep vein thrombosis; and

(d) Patient D had non-Hodgkin's lymphoma.

The relevance and possible aetiology of anti-golgi antibodies are also discussed.

Keywords: Anti-golgi antibodies, virus-induced autoimmunity

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INTRODUCTION

In 1982, Louvard et al⁽¹⁾ immunized rabbits with enriched golgi membrane fractions and, after several steps of purification, obtained rabbit antisera that was confirmed by immunoelectron microscopy to be anti-golgi in specificity. Interestingly, when tested by indirect immunofluorescence on a variety of tissue-culture cells, these anti-golgi antibodies (AGA) lighted up a distinct perinuclear crescent shaped structure.

A few months later, Rodriguez et al⁽²⁾ reported a similar immunofluorescence pattern, but this time the source of the AGA was the serum of a patient with Sjogren's syndrome and lymphoma. This was the first case-report of a patient with AGA. The anti-golgi specificity was confirmed by histochemical studies and by the demonstration that pre-absorption of the patient's sera against isolated golgi cisternae abolished the distinct perinuclear fluorescence.

In 1984, Fritzler et al⁽³⁾ reported 8 patients with AGA: 6 had systemic lupus erythematosus, one had Sjogren's syndrome and one had hepatitis. Two further reports of AGA were published in 1988:

(a) Blaschek et al⁽⁴⁾ described 2 patients with Sjogren's syndrome; and

(b) Gaspar et al⁽⁵⁾ described a patient with idiopathic late-onset cerebellar ataxia who was found to have AGA in both his serum and his cerebrospinal fluid.

Our laboratory receives specimens for the detection of antinuclear antibodies (by indirect immunofluorescence) on a

routine basis. This study describes 4 patients whose sera exhibited immunofluorescence staining suggestive of AGA activity, without any anti-nuclear staining.

MATERIALS AND METHOD

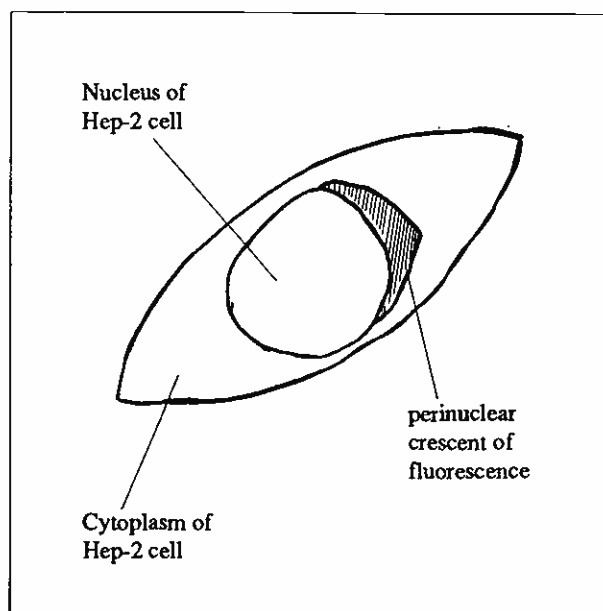
The anti-nuclear fluorescence test is routinely performed in our laboratory. Briefly, the indirect immunofluorescence test utilizes Hep-2 cell lines purchased from Kallestad Labs, Texas, as the substrate. Patients' sera were screened at an initial serum dilution of 1:40 in phosphate buffered saline (PBS). The serum was left to react with the Hep-2 substrate for 20 minutes at room temperature. After rinsing in PBS, fluorescein-conjugated anti-human immunoglobulin was added for 20 minutes at room temperature. After a second rinse in PBS, the slides were mounted and examined under UV-illumination for specific fluorescent patterns.

Patients whose sera exhibited staining patterns suggestive of AGA had their medical records reviewed retrospectively.

RESULTS

Four cases were noted to exhibit a characteristic perinuclear crescent of fluorescence without any staining in the nucleus nor anywhere else in the cytoplasm of the Hep-2 cells. Repeat

Fig 1- The perinuclear crescent of fluorescence typical of anti-golgi antibodies.



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tests with these 4 sera samples reproduced identical fluorescence patterns. (Fig 1)

Patient 1

A 20-year-old Chinese male presented with oedema in his lower limbs and face. He had had upper respiratory tract infection two weeks prior to this admission. Clinically, he was hypertensive (BP 180/110) with facial and lower-limb oedema. Laboratory investigations were consistent with a diagnosis of acute glomerulonephritis. He was not evaluated for any other autoantibodies. He was treated for his hypertension and discharged when well.

Patient 2

A 27-year-old Chinese male presented with a history of low-grade fever and right hypochondrial pain. He was noted to have a tinge of jaundice and had slight tenderness over his enlarged liver. Laboratory investigations were consistent with a diagnosis of acute viral hepatitis. He was managed conservatively and recovered. No other autoantibodies were investigated for.

Patient 3

A 77-year-old Chinese female presented with a 3-day history of right leg swelling. She also had a previous history of hypertension and stroke. On examination, warmth and tenderness was noted over her right calf. She had venogram evidence of deep vein thrombosis. She was started on anti-hypertensives and warfarin treatment. She was negative for antibodies to double-stranded DNA, VDRL and hepatitis serology. She recovered uneventfully and was subsequently discharged.

Patient 4

A 52-year-old Malay male presented in 1986 with fever and loss of both weight and appetite. On examination, he had a tender 4cm liver. Physical findings and laboratory investigations were suggestive of a liver abscess. He was negative for anti-amoebic antibodies. He had percutaneous drainage done and eventually was discharged on recovery. He was not screened for anti-nuclear fluorescence during this admission.

Three years later, he returned with right hypochondrial pain. He was found to have an irregularly enlarged liver and thrombocytopenia. Bone marrow aspirate revealed lymphomatous infiltrates. He was diagnosed to have non-Hodgkin's lymphoma and received chemotherapy. During this admission screening for anti-nuclear fluorescence revealed AGA.

DISCUSSION

The fluorescence patterns observed in all 4 cases were typical in appearance and location of AGA. No other autoantibody has ever been shown to cause a similar fluorescence pattern. Several tests may be done to confirm their anti-golgi specificity:

- (a) immunoelectron microscopic verification;
- (b) absorption with isolated golgi cisternae and repeating the test to demonstrate the disappearance of the typical perinuclear crescent;
- (c) treatment of the Hep-2 cells with chemicals that are known to disrupt the golgi apparatus (eg. colcemid, taxol or monensin), followed by a repeat test to demonstrate a corresponding disruption of the perinuclear crescent;
- (d) immunoblotting experiments with the sera being screened against blots of membrane fractions of the golgi cisternae; and
- (e) treatment of the Hep-2 cells with thiamine pyrophosphatase, which is known to selectively stain the golgi⁽⁶⁾, to demonstrate a staining pattern indistinguishable from that observed with our patients' sera.

Though the majority of the patients described in the literature to have AGA, had autoimmune diseases, none in our

series had lupus nor Sjogren's syndrome. The report of a patient with lymphoma⁽²⁾ and another with hepatitis⁽³⁾ parallel our findings. Sera for antinuclear fluorescence tests are invariably received from patients with a probable diagnosis of autoimmune disease. It is possible this skews the detection of AGA towards patients with autoimmune-diseases. Interestingly, 5 out of the 8 patients described by Fritzler⁽⁹⁾ also had liver disease. In our study, 2 out of the 4 cases also had liver pathology. However, what this all may mean, still remains vague. The fact that a variety of disease states may be associated with the presence of AGA complicates the search for its aetiology.

A wide variety of other autoantibodies directed to antigens present in the nucleus, cytoplasm and plasma membranes of cells in different tissues have been described. A subset of these have been found to be of value in the diagnosis and follow-up of specific diseases. Some autoantibodies have been shown to have direct roles in disease pathogenesis, as in diseases with anti-receptor antibodies and diseases marked by complement binding immune-complexes. On the other hand, some autoantibodies have been considered to be epiphenomena secondary to tissue damage or injury induced by chemical or biological agents. The aetiology and significance of the AGA is as mysterious today as it was 8 years ago, when first described.

Fortunately, some hints have surfaced. In 1984, Weiland et al⁽⁷⁾ reported the detection of an autoantibody activity against the evolutionary conserved antigen associated with the golgi complex in mice bearing a progressive Moloney sarcoma virus transformant. A year later, the same authors demonstrated⁽⁸⁾ that it was not the tumour itself that was responsible for the induction of AGA; but these mice harboured in their sera a transmissible agent capable of inducing AGA formation in newly injected mice. After 2 years of biochemical and electron-microscopic studies, Weiland et al⁽⁹⁾ reported "lactate dehydrogenase-elevating virus induces anti-golgi apparatus antibodies".

They postulated 4 possible pathogenetic mechanisms:

- a. the virus could cause general polyclonal B-cell activation, one of the results of which is the increased production of AGA;
- b. cytotoxic replication of the virus in macrophages may result in the release of some self-antigens that stimulate autoantibody response;
- c. morphogenesis of this virus occurs in the golgi apparatus by budding into vacuoles. This, perhaps, leads to combination of viral nucleic acid with constituents of the golgi organelle, causing the cellular protein to be considered foreign; or
- d. antigen mimicry between the novel virus and antigenic epitopes on the golgi might be responsible for triggering autoimmune responses.

It is interesting to note that these mice never exhibited any clinical features suggestive of autoimmunity. Several reasons may account for this. Firstly, the mice may have had subclinical features of autoimmune disease, masked by the underlying malignant disease. Secondly, additional contributory factors present in man but not in mice may be required to precipitate clinical disease. Finally, the observed AGA may represent nothing more than epiphenomena caused by pathogenetic mechanisms postulated by Weiland et al, as listed above.

Virus-induced autoimmunity has been documented for reovirus⁽¹⁰⁾ and coxsackie virus⁽¹¹⁾; likewise, the spectrum of diseases characterized by the appearance of AGA may represent yet another example of virus-induced autoimmunity.

In the next couple of years we are likely to learn more about this novel virus and the antigenic make-up of the golgi apparatus. Till then, the origin and significance of the AGA will still remain an unsolved mystery.

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