*Acta Medica Okayama*

*Volume* 37, *Issue* 6 1983 *Article* 8

DECEMBER 1983

# A simplified quantification method of complex-release activity using peroxidase as immune complex antigen.

Tetsuki Amano* Yasushi Aibara† Norio Kuwajima‡

Sinya Suzuki** Zensuke Ota††

*Okayama University,

†Okayama University,

‡Okayama University,

**Okayama University,

††Okayama University,

Copyright ©1999 OKAYAMA UNIVERSITY MEDICAL SCHOOL. All rights reserved.

# A simplified quantification method of complex-release activity using peroxidase as immune complex antigen.*

Tetsuki Amano, Yasushi Aibara, Norio Kuwajima, Sinya Suzuki, and Zensuke Ota

**Abstract**

The complement-mediated solubilization of precipitable immune complexes (complex-release activity) in serum specimens was determined by a simplified method using peroxidase as an immune complex antigen. The results correlated well with the hemolytic activity via the classical complement pathway and that via the alternative complement pathway. This simplified method proved to be reliable and useful.

**KEYWORDS:** solubilization of immune complex, complement, peroxidase

*PMID: 6364698 [PubMed - indexed for MEDLINE]
Copyright (C) OKAYAMA UNIVERSITY MEDICAL SCHOOL

Acta Med. Okayama 37, (6), 519-520 (1983)

## — BRIEF NOTE —

# A SIMPLIFIED QUANTIFICATION METHOD OF COMPLEX-RELEASE ACTIVITY USING PEROXIDASE AS IMMUNE COMPLEX ANTIGEN

Tetsuki AMANO, Yasushi AIBARA, Norio KUWAJIMA, Shinya SUZUKI and Zensuke OTA

*Third Department of Medicine, Okayama University Medical School, Okayama 700, Japan*

*Received July 18, 1983*

*Abstract.* The complement-mediated solubilization of precipitable immune complexes (complex-release activity) in serum specimens was determined by a simplified method using peroxidase as an immune complex antigen. The results correlated well with the hemolytic activity via the classical complement pathway and that via the alternative complement pathway. This simplified method proved to be reliable and useful.

*Key words :* solubilization of immune complex, complement, peroxidase.

The complement-mediated solubilization of precipitable immune complexes (IC), *i.e.*, complex-release activity (CRA) (1), may influence the pathogenesis of autoimmune disease. We have developed a simplified method to measure CRA and examined its usefulness using 63 fresh serum samples obtained from 30 patients with systemic lupus erythematosus, 19 patients with rheumatoid arthritis and 14 healthy individuals.

For preparation of precipitable IC, equal amounts of horseradish peroxidase (PO) (Wako-Junyaku, Osaka) and anti-PO rabbit IgG (Cappel Lab., USA) were incubated at equivalence for 1 h at 37 ℃, kept overnight at 4 ℃, and centrifuged for 15 min at 3000 rpm. The resulting sediment was washed and suspended in 0.02 M phosphate buffered saline solution (PBS) (pH 7.4). This solution was designated as PO-IC and adjusted to the concentration of 11 $\mu$g/ml.

For complement-mediated solubilization of PO-IC, 0.2 ml of precipitable PO-IC and 0.1 ml of the serum to be examined were incubated for 1 h at 37 ℃ in a test tube, and then centrifuged for 15 min at 3000 rpm after adding 1 ml PBS. The resulting supernatant (0.5 ml) and 0.4 ml of 0.2 % 5-aminosalicylic acid containing 0.016 % $H_2O_2$ were incubated for 1 h at 37 ℃. The reaction was stopped by adding 0.1 ml lN-NaOH, and 3 ml PBS was added. The absorbance at O.D. 450 nm was regarded as the CRA-value.

For CRA via the alternative complement pathway, PO-IC and the sera to be examined were incubated with 0.015 M ethylene glycoltetraacetic acid. The ab-

TABLE. CORRELATION COEFFICIENTS BETWEEN CRA AND CH50, ACH50 AND ACRA IN VARIOUS SERA.

| | CH50 | ACH50 | ACRA |
|---|---|---|---|
| r | 0.79 | 0.65 | 0.89 |
| p< | 0.001 | 0.001 | 0.001 |
| n | 63 | 62 | 63 |

sorbance was measured as for the CRA, and expressed as ACRA.

Hemolytic activity via the classical complement pathway (CH50) and that via the alternative complement pathway (ACH50) were measured according to Mayer's method (2) and our method using rabbit red blood cells (3), respectively.

As shown in Table, CRA correlated well with CH50, ACH50 and ACRA in the 63 fresh serum samples examined.

We have devised a simplified technique for measuring the solubilization of IC using PO as an antigen. CRA was shown to correlate well not only with ACH50 but also CH50. CRA, mainly mediated by six components of the alternative complement pathway (4), are enhanced by the activation of the classical complement pathway (5). Therefore, this newly devised method should be useful for measuring the solubilization of IC by complement.

## REFERENCES


1. Miller, G.W. and Nussenzweig, V.: A new complement function : Solubilization of antigen-antibody aggregates. *Proc. Natl. Acad. Sci. U.S.A.* **72,** 418-422, 1975.
2. Mayer, M.M.: Complement and complement fixation. In *Experimental Immunochemistry,* 2nd Ed. ed. E. A. Kabat and M.M. Mayer, C.C. Thomas, Springfield, Illinois, pp. 133-240, 1964.
3. Amano, T., Yoshinouchi, T., Miyajima, H., Mitsuhashi, Y. and Ofuji, T.: Alternative pathway in SLE. *Clin. Immunol.* **8,** 289-297, 1976 (in Japanese).
4. Fujita, T., Takata, Y. and Tamura, N.: Solubilization of immune precipitates by six isolated alternative pathway proteins. *J. Exp. Med.* **154,** 1743-1751, 1981.
5. Takahashi, M., Takahashi, S. and Hirose, S.: Solubilization of antigen antibody complexes : A new function of complement as a regulator of immune reactions. *Prog. Allergy* (Karger, Basel). **27,** 134-166, 1980.