IMMUNOENHANCING EFFECTS OF CONJUGATED LINOLEIC ACID ON PHAGOCYTIC RESPONSE IN THE PIGS

Ju-hyang Kim[1], In-kyung Ko[1], Chung-soo Chung[2], Chul-young Lee[3], and Mhan-pyo Yang[1]

[1]Laboratory of Veterinary Internal Medicine, College of Veterinary Medicine and Research Institute of Verterinary Mendicine, [2]Department of Animal Science, Chungbuk National University, Cheongju, Chungbuk 361-763, [3]Department of International Livestock Industry, Chinju National University, Chinju 660-758, Republic of Korea

Introduction

An anti-mutagenic activity in hamburger was discovered in 1979[1]. This anti-mutagenic extract was shown to prevent 7,12-dimethylbenz[a]anthracene-induced mouse epidermal tumors[1,2]. The active substance with anti-mutagen and anti-carcinogen was shown to be a mixture of structural and geometrical isomers of linoleic acid containing conjugated double bonds[2]. Conjugated linoleic acid (CLA) consists of sixteen possible geometric isomers (9c-11c, 9c-11t, 9t-11c, 9t-11t, 10c-12c, 10c-12t, 10t-12c, 10t-12t, 7t-9c, 7t-9t, 11t-13c, 11t-13t, 12c-14t, 12t-14t, 8t-10c, 8t-10t CLA), two of which (9c-11t, 10t-12c CLA) are known to possess biological activity. CLA is presently under research because of its potent anticarcinogenic effects and on the immune system. Especially, CLA increased mitogen-induced lymphocyte blastogenesis and murine macrophage killing ability[3]. Lymphocytes from CLA-supplemented animals had increased IL-2 production and no change in lymphocyte cytotoxicity, IL-1 and PGE_2 production, or DTH reaction[4]. However, immunomodulatory effect of CLA was not investigated. In the present study, the *in vitro* effects of several CLA isomers (10t-12c, 9c-11t, 9c-11c, 9t-11t and CLA mixture containing 44% 10t-12c) on phagocytic response of peripheral blood mononuclear cells (MNC) and polymorphonuclear cells (PMN) in adult pigs were examined.

Methods and Materials

Blood was collected in heparinized tubes from anterior vena cava of healthy pigs, average 1-year old. The MNC and PMN were isolated as described previously[5]. To examine the effect of CLA on phagocytic activity of porcine blood phagocytes, freshly isolated MNC and PMN were cultured with CLA at different concentration for 12 hours. The cultures were thereafter supplemented with FITC-labelled latex for the final 1 hour. After washing, the phagocytized latex cells per total 10,000 cells

were immediately estimated by a flow cytometry. Phagocytic response of porcine MNC and PMN to culture supernatant from MNC treated with CLA for 24 hours was also examined in the same manner. The Student's *t* test was used for statistical significance determinations.

Results and Discussion

CLA at higher concentration of 50 to 200 μM exhibited a low viability of cells by trypan blue exclusion. Thus, CLA were used at concentration of 20 μM showing no cytotoxic effect and high cell viability. The *in vitro* direct treatments of CLA (10 to 20 μM) have a weak or negligible effect on phagocytosis of PMN as well as MNC composed of approximately 10% monocytes and 90% lymphocytes. These findings indicate that CLA themselves were not active in the phagocytic response of phagocytes.

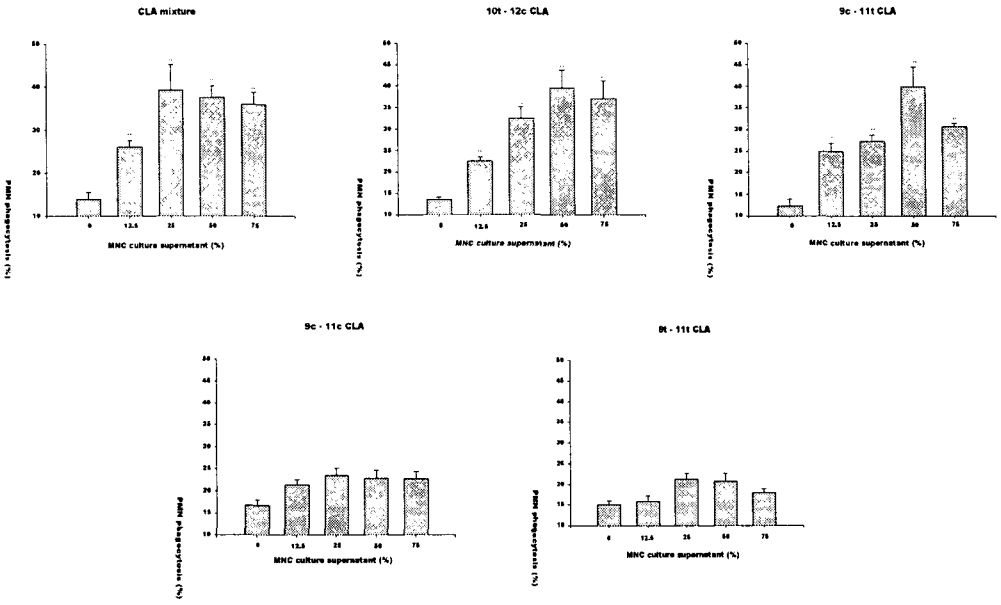

Fig. 1. Phagocytic response of porcine PMN to culture supernatant from MNC treated with 20μM of CLA (10t-12c, 9c-11t, 9c-11c, 9t-11t and CLA mixture). The data represent mean ± SEM(n=4). **P<0.01, compared to control (0).

The phagocytic activity of MNC was not enhanced by culture supernatant from MNC ($2x10^6$ cells/ml) treated with 20 μM CLA for 24 hours. As shown in Fig. 1, however, the phagocytic activity of PMN in the same procedure was remarkably enhanced by culture supernatant from MNC but not PMN treated with CLA (10t-12c, 9c-11t and CLA mixture but not 9c-11c and 9t-11t).

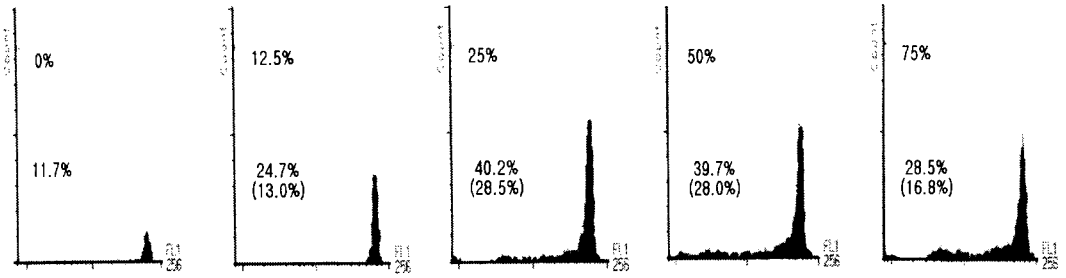

Fig. 2. Flow cytometric profile of phagocytized cells in porcine PMN to culture supernatant (12.5 to 75%) from MNC treated with CLA mixture (20μM). Numbers in parenthesis indicated net phagocytic acitivity calculated by reducing the value of untreated cells from that of treated cells.

The representative phagocytic activity of PMN to culture supernatant from MNC treated with CLA mixture was shown in Fig. 2. The supplement of the culture supernatant from MNC treated with CLA to monocytes-riched cells fractionated from dot plot profile in flowcytometric cytography also resulted in the enhancement of their phagocytic responses (Fig.3).

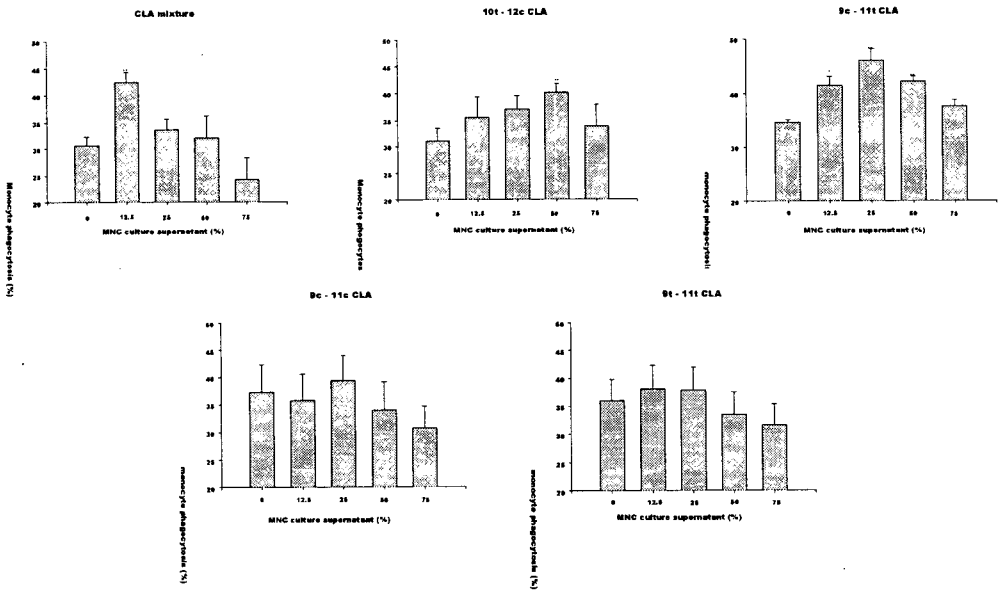

Fig. 3. Phagocytic response of porcine monocyte-riched fraction to culture supernatant from MNC treated with 20 μM of CLA (10t-12c, 9c-11t, 9c-11c, 9t-11t and CLA mixture). The data represent mean ± SEM(n=4). **P<0.01, compared to control (0 %).

These results strongly suggest that CLA has an enhancing effect on phagocytosis of PMN and monocytes, which may be mediated through active humoral substances produced by CLA-stimulated

MNC. It is also assumed that CLAs act on MNC to produce active soluble products which are involved in the enhancement of PMN phagocytosis.

Reference

1. Mark E.C. and Michael W.P. (1998) Int. Dairy Journal. 8, 459.
2. Michael W.P., Yeonhwa P. and Mark E.C. (2001) Progress in Lipid Research. 40, 283.
3. Michael G.H., Sung-Nim H., Dayong W., Bruce A.W., Mohsen M., Jodee L.D., Donald E.S. and Simin N.M. (1999) J.Nutr. 129, 32.
4. Leah D.W., Mark E.C. and Richard L.A. (2000) Pharmacological Research. 42(6), 503.
5. Se-hyung P., Young-Won Y. and Mhan-pyo Y. (1998) Korean J. Vet Clin Med. 15(1), 104.