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Chitosan augments cytolytic activity of mouse lymphocytes

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Abstract

The effect of chitosan on cytolytic activity of mouse lymphocytes was examined. Chitosan augmented NK and LAK activity of lymphocytes prepared from C3H/HeJ mice in a dose-dependent manner. Optimum dosage of chitosan was found to be at 64 μg/ml in the case of NK activity, but augmentation of LAK activity was still increasing at 96 μg/ml.

Key words chitosan, cytolytic activity, NK activity, LAK activity.

Abbreviations NK, natural killer ; LAK, lymphokine activated killer ; FBS, fetal bovine serum ; HEPES, N-2-hydroxylethylpiperazine-N'-ethanesulfonic acid ; IL-2, interleukin-2.

Introduction

Chitosan is a polysaccharide derived from chitin which is a main component of the protective cuticles of crustacea and insects, and also occurs in the cell walls of some fungi and microorganisms. It has been reported that chitosan exerts various beneficial effects on the health such as hypocholesterolemic, wound healing and immunoenhancing effects.[1-3]

Chitosan is expected to augment NK activity because D-glucosamine, which is a monomeric form of chitosan, has been shown to augment it.[4] The purpose of the present research is to clarify the effect of chitosan on cytolytic activity of mouse lymphocytes toward cancer cells.

Materials and Methods

Materials : Chitosan used in this experiment was 81 % deacetylated chitin which was kindly provided by Ueno Fine Chemicals Industry LTD. Chitosan (0.6 g) was suspended in 40 ml of distilled water, and stirred over night at 0 °C. Insoluble material was removed by centrifugation at 12,000×g for 10 min. Chitosan concentration of the resulting supernatant was determined to be 128 μg/ml by lyophilization. Then the supernatant was sterilized by filtration and mixed with concentrated culture medium and water to make 25, 50 and 75 % of saturated chitosan solution in the medium. This diluted chitosan solution was used in the experiments.

Preparation of effector cells : Spleens were removed from C3H / HeJ mice. The organs were gently teased by means of dissecting forceps, and allowed to sediment, and the cell suspensions were aspirated and transferred to sterile tubes. Then splenocytes were suspended in Tris-buffered ammonium chloride solution to lyze red blood cells. The resulting lymphocyte preparations were washed by centrifugation in RPMI 1640 medium supplemented with 25 mM HEPES, 25 mg/ml gentamycin and 10 % FBS. Then the lymphocytes were cultured for three days in the above medium with various concentrations of chitosan in the presence or absence of 20 U/ml IL-2 (mouse, recombinant).

Cytolytic assay : The chromium release assay[5] was used to evaluate cytolytic activity of the effector cells for YAC-1 (mouse lymphoma) target cells. Briefly, YAC-1 cells (4×10^6 cells) suspended in the medium were labeled with 100 μ Ci of Na $^{51}CrO_4$ for 90 min at 37°C , washed three times. Effector cells were adjusted to varying concentrations and added to 1.5×10^4 of ^{51}Cr-labeled YAC-1 cells. Effector and target cell

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mixture (200 μl) at ratio 25 or 50 to 1 were dispersed as multiplicate samples into individual wells of a 96-well microtiter plate and centrifuged at 50×g for 5 min. After incubation for 4 h in a humidified CO_2 incubator, the plates were again centrifuged, and 100 μl of supernatant were collected and measured for radioactivity in a γ-counter. Spontaneous release was determined by measuring ^{51}Cr release of labeled target cells in the absence of target cells. Maximal release was determined by exposure of labeled target cells to 0.05 % Triton X-100. The percentage of cytolytic activity was computed from specific ^{51}Cr release by the following fomula.

$$\% \text{ of cytolytic activity} = 100 \times \frac{\text{cpm experimental} - \text{cpm spontaneous}}{\text{cpm maximal} - \text{cpm spontaneous}}$$

Results

NK and LAK activities of mouse lymphocytes were augmented dose dependently by chitosan at E/T ratio 50 (Fig. 1). Essentially the same results were observed at E/T ratio 25 (data not shown). Significant augmentation of NK activity was observed from 32 μg/ml, and optimum augmentation was at 64 μg/ml. At this concentration, NK activity was augmented over two fold higher than 0 μg/ml (4.5 % and 11.43 % at 0 and 64 μg/ml, respectively). On the other hand, significant augmentation of LAK activity was observed from 64 μg/ml, and the rate of augmentation was still increasing at 96 μg/ml.

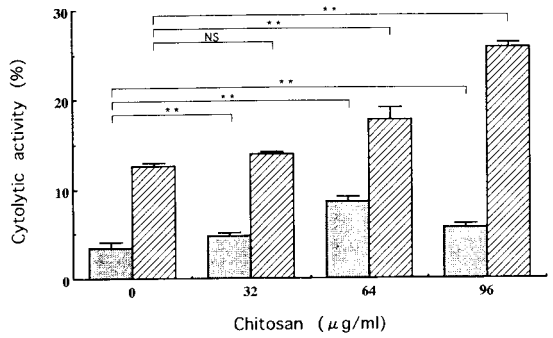

Fig. 1 Effect of chitosan on cytolytic activity of mouse lymphocytes using E/T ratio 50.
Effector cells were cultured for three days with various concentrations of chitosan in the sbsence (▨, NK activity) or presence (▨, LAK activity) of IL2 (20 U/ml). Values are means±S.E. (n=8). Vs. coresponding control : NS, not significant ; **, $p<0.01$.

Discussion

NK cells emerge as an important mechanism in natural resistance against cancer and infectious diseases.[6,7] These cells can promptly destroy a variety of malignant cells without any deliberate immunization or arming with antibody.[8] Moreover, cytocidal activity of NK cells can be induced against NK-resistant and substantially augmented against NK-susceptible tumor cells by IL-2.[9-11]

In the present experiment, it was found that chitosan augmented NK and LAK activities of lymphocytes dose dependently. The mechanism of enhancement by chitosan may be the similar way of glucosamine which perturbs thymidine metabolism.[1] The dose response of augmentation toward NK activity by chitosan was different from that toward LAK activity. Although LAK precursor cells are not well defined,[12,13] these results suggest that LAK cells acquire different characteristics from NK cells through the induction by IL-2.

As chitosan is high molecular compound (around several millions of estimated molecular weight), it might not be absorbed directly from the intestine. Intraepitherial lymphocytes (IEL) in mammalian intestinal mucosa have been known to have NK and LAK activities,[14,15] and infiltrate to lumen. Therefore, it seems possible that chitosan enhances directly cytolytic activity of IEL in the lumen. Thus chitosan may act in defense against cancer and microbial diseases in the gastrointestinal region. Further experiments are needed to prove this hypothesis.

和文抄録

マウスリンパ球の細胞障害活性に及ぼすキトサンの影響を調べた。キトサンは，C3H/HeJ マウスリンパ球の NK 及び LAK 活性を濃度依存的に増強した。その至適濃度は，NK 活性に対しては 64 μg/ml で観測されたが，LAK 活性では 96 μg/ml においてもなお増強していた。

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