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L-arginine supplementation does not affect chemically induced carcinogenesis and tumor growth in BALB-c mice

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Go to:

Abstract

T-cell ζ-chain downregulation is common in various types of cancer and it is proposed as a mechanism of cancer immunosubversion. L-arginine consumption by arginase rich suppressor myeloid cells has been incriminated. The effect of L-arginine supplementation on chemically induced carcinogenesis and tumor growth in mice was evaluated.

Methods: Eight-week old female BALB-c mice were used. Ten mice were injected i.m. with 0.6 mg methylcholanthrene (MCA) once. Ten mice were injected with MCA once and were receiving L-arginine supplementation (5% in animal drinking water) continuously during the study. Mice with cancer were sacrificed 12 weeks after.

Results: From the 10 MCA injected mice 6 developed sarcoma. From the 10 MCA injected mice that were receiving L-arginine supplementation 7 developed sarcoma. L-arginine supplementation did not affect MCA induced carcinogenesis (p=1.0, Fisher's exact test). The weight of tumors was not different between the tumors derived from mice that were or were not receiving L-arginine supplementation (1088.3±590.2 mg vs. 969.6±608.1 mg respectively, p=0.729, unpaired t-test).

Conclusion: L-arginine supplementation does not affect chemically induced carcinogenesis and tumor growth in BALB-c mice. Although ζ-chain downregulation could be a mechanism of cancer immunosubversion there are enough other cancer immunosubversion mechanisms that were not overwhelmed by L-arginine supplementation. Additionally, except cancer immunosubversion, cancer immunoselection is another, possibly more significant, mechanism of tumor escape from immunosurveillance.

Keywords: Cancer, immune surveillance, zeta-chain, L-arginine

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Numerous innate and adaptive immune effector cells and molecules participate in the recognition and destruction of cancer cells, a process that is known as cancer immunosurveillance. But cancer cells avoid such immunosurveillance through the outgrowth of poorly immunogenic tumor-cell variants (immunoselection) and through subversion of the immune system (immunosubversion) [1].

One relatively resent proposed mechanism of immunosubversion in cancer is the downregulation of the T-cell ζ-chain. Zeta chain is a stable constituent of the antigen specific T-cell receptor and its

phosphorylation is one of the earliest and key events in the T-cell signal transduction [2]. Consequently, ζ-chain downregulation could result in a deficient T-cell immune response. In many types of human cancer T-cell ζ-chain expression as well as T-cell immune response are impaired[2,3].

L-arginine plays a key role in ζ-chain downregulation. Firstly, it was observed that Jurkat T-cells cultured in arginine depleted medium had a rapid decrease in ζ- chain expression due to the shorter ζ-chain mRNA half life[4]. Later, cell culture experiments showed that increased arginase I expression in activated macrophages decreased L-arginine availability in the medium leading to T-cell ζ-chain downregulation. A possible mechanism for T-cell dysfunction and ζ-chain downregulation in cancer and chronic inflammation was proposed[5]. The role of L-arginine in ζ-chain downregulation in cancer was confirmed using a 3LL murine lung carcinoma model. A subpopulation of mature tumor-associated myeloid cells expressed high levels of arginase I, whereas tumor cells and infiltrating lymphocytes did not. Decreased ζ-chain expression and impaired T-cell function were detected too. In cell cultures L-arginine depletion by tumor-associated myeloid cells blocked the re-expression of ζ-chain in stimulated T cells and inhibited antigen-specific proliferation. The injection of an arginase inhibitor blocked growth of subcutaneous 3LL lung carcinoma in mice. Interestingly, high levels of arginase I were also found in tumor samples of patients with non-small cell carcinoma[6]. The existence of suppressor myeloid cells producing arginase in human cancer patients was confirmed for the first time in renal cell carcinoma. The increased arginase activity was limited to a specific subset of CD11b+, CD14-, CD15+ cells with a polymorphonuclear granulocyte morphology and markers, instead of macrophages or dendritic cells described in mouse models[7].

The aim of the present study was to evaluate the effect of L-arginine supplementation on chemically induced carcinogenesis and tumor growth in mice. Horiguchi et al showed that mice bearing primary methylcholanthrene (MCA) induced tumors had significantly diminished T-cell function, impaired capacity to produce Th1 cytokines, and markedly reduced levels of the signal-transducing ζ-chain in T cells, similar to that described in cancer patients. In contrast, T cells from mice bearing rapidly growing transplanted tumors were only marginally affected. These findings could explain the differences in the high efficacy of immunotherapy in mice with transplanted tumors and the low therapeutic results in cancer patients[8].

Go to:

Methods

Eight-week old female BALB-c mice bred and maintained at the animal facilities of the Research Institute at the Theagenion Anticancer Hospital of Thessaloniki were used. Four groups of ten animals each were formed. The first group was used as control. The second group received only L-arginine supplementation. The third group was injected with MCA once. Finally, the forth group was injected with MCA once and was receiving L-arginine supplementation continuously during the study.

L-arginine (Sigma-Aldrich, St. Louis, MO) was administered orally. For this purpose a 5% L-arginine solution in the animal drinking water was used everyday, one week before the carcinogen injection and until the end of the study. Because the above L-arginine solution is very alkaline (pH>11) an HCl solution was used in order to achieve a relatively neutral solution (pH=7.4).

Methylcholanthrene (Sigma-Aldrich) (0.6mg dissolved in 0.1ml of corn oil) was injected intramuscularly into the muscles of the upper thigh of hind limb of the mice once[8]. Afterwards the mice were inspected weekly.

Go to:

Results

As it was expected cancer was not developed in the groups of mice that received only water or L-arginine supplementation. In some animals of the other groups cancer was started to be developed from the 4th week after the MCA injection.

At the 12th week after the MCA injection the animals that developed cancer were sacrificed, whereas the others were inspected for another 8 weeks period. None of the animals that were injected with MCA and remained without cancer until the 12th week developed cancer during the additional 8 weeks of observation. Pathological examination of the tumors set the diagnosis of sarcoma.

From the 10 MCA injected mice 6 (60%) developed cancer. From the 10 MCA injected mice that were receiving L-arginine supplementation 7 (70%) developed cancer. L-arginine supplementation did not affect MCA induced carcinogenesis (two sided p=1.0, Fisher's exact test) (Fig. 1).

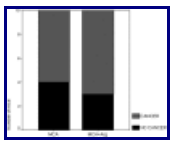

Figure 1.
The effect of L-arginine supplementation on methylcholanthrene induced carcinogenesis
Regarding tumor growth, the weight of the tumors did not differ significantly between the two groups. It was 969.6±608.1 mg in MCA injected mice and 1088.3±590.2 mg in MCA injected mice that were receiving L-arginine supplementation (two sided p=0.729, unpaired t-test) (Fig. 2).

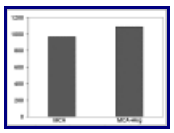

Figure 2.
The effect of L-arginine supplementation on methylcholanthrene induced tumor growth
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Discussion

The aim of the present study was to evaluate the effect of L-arginine supplementation on chemically induced carcinogenesis and tumor growth in mice. The rational was to overwhelm the L-arginine consumption by the rich in arginase I suppressor myeloid cells preventing the subsequent T-cell ζ-chain downregulation[6,7]. It was expected that the prevention of ζ-chain downregulation improving immune response would increased cancer immunosurveillance leading to decreased carcinogenesis and/or in case of cancer development to retardation of tumor growth.

Reynolds et al in a relative old study showed that Larginine supplementation (1% in the animal drinking fluid) in mice bearing transplanted tumors improved immune function. In that study L-arginine supplementation was promising since it did not affected carcinogenesis but it retarded tumor growth[9]. In contrary, the results of the present study were disappointing. Regarding MCA induced carcinogenesis, from the 10 MCA injected mice 6 (60%) developed sarcoma. From the 10 MCA injected mice that were receiving L-arginine supplementation 7 (70%) developed sarcoma. L-arginine supplementation did not affect MCA induced carcinogenesis. As regards the effect of L-arginine supplementation on tumor growth, the weight of the MCA induced tumors was not different between the tumors derived from mice that were or were not receiving L-arginine supplementation.

It should be noted that there are key differences between the present study and the study by

Reynolds at al. First, the tumors examined were pathologically different and it is well known that different types of cancer have different biological behaviour. Second, the tumors examined by Reynolds et al were transplanted tumors. In the present study chemically induced primary tumors were examined[9]. On the other hand Horiguchi et al showed that mice bearing primary MCA induced tumors have significantly diminished T-cell function and markedly reduced levels of the signal-transducing ζ-chain in T cells, similar to that described in cancer patients. In contrast, T cells from mice bearing rapidly growing transplanted tumors are only marginally affected. The authors attributed this dissimilarity in the different tumor growth rate in primary chemically induced cancer and in transplanted cancer. Finally, they hypothesized that these findings could explain the differences regarding the high efficacy of immunotherapy in mice with transplanted tumors and the low therapeutic results in cancer patients[8]. According to the study by Horiguchi et al, one could expect that in the model used in the present study, L-arginine supplementation should have beneficial effect. But it did not. Of course, the different biological behaviour of different types of cancer and certainly of different species prevents universal conclusions form single studies about effective immunotherapeutic intervention in cancer.

From the results of the present study it could be hypothesized that ζ-chain downregulation is simply an epiphenomenon of cancer that does not play a significant role in cancer immunosurveillance. A more rationale hypothesis is that although ζ-chain downregulation could be a mechanism of cancer immunosubversion there are enough other cancer immunosubversion mechanisms[1] that were not overwhelmed by L-arginine supplementation. Additionally, except cancer immunosubversion, cancer immunoselection is another, possibly more significant, mechanism of tumor escape from immunosurveillance[1]. Consequently manipulating only one component of the immune system it is not likely to lead in an effective immune response against cancer.

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