Surg Today (2003) 33:655–661
DOI 10.1007/s00595-002-2563-2

SURGERY TODAY

Elevated Serum Arginase Activity Levels in Patients with Breast Cancer

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Abstract

Purpose. Recently, the high activity of arginase enzyme has been observed in the sera of malignant neoplasms. In this pathogenic condition, it is said that arginase strongly inhibits lymphocyte proliferation and plays a role in providing ornithine as a substrate for biosynthesis of polyamines, which have been found in various types of cancer. The aim of this study was to examine the arginase activity levels in breast cancer as a marker.

Methods. We evaluated the serum arginase activity levels in 48 females with breast cancer, in 30 females with benign disease, and in 50 healthy control subjects. The serum arginase activities were determined according to the slightly modified method of Chinard.

Results. The mean activity of arginase was found to be high in the early stages ($n = 27$, stage I + II, $P < 0.01$), and higher in the advanced states ($n = 21$, stage III + IV, $P < 0.001$) of the malignant group in comparison with those of the normal subjects.

Conclusion. A high arginase level in breast cancer was observed to possibly be released into the serum: namely, the more advanced the breast cancer, the higher the serum level of arginase enzyme activity. Therefore, this enzyme might serve as a useful biological marker in breast cancer while also being an indicator of breast cancer progression.

Key words Breast cancer · Tumor marker · Enzyme · Arginase activity

Reprint requests to: M.F. Polat
Received: March 12, 2002 / Accepted: November 19, 2002

Introduction

Breast cancer is a leading cause of mortality and morbidity among women in modern society.[1] In addition to palpation, mammography, and similar procedures, a series of tumor markers that are not specific for breast cancer were recently measured for screening, early detection, progression, and a preoperative evaluation of breast tumors. These markers include carcinoembryonic antigen (CEA), the carcinoma antigens CA 15-3, CA 549, mucinous carcinoma-associated antigen (MCA), total sialic acid (TSA), lipid-bound sialic acid (LSA), and estradiol or progesterone receptors.[2–8]

Arginase (L-arginine amidinohydrolase; EC 3.5.3.1), the final enzyme of the urea cycles, catalyzes the hydrolysis of arginine to urea and ornithine.[9] Arginase enzyme exists abundantly in the human liver and in trace amounts in other organs such as the kidney, brain, and intestine.[10] The function of extrahepatic arginase enzyme is essentially unknown and this enzyme may be involved in cell proliferation.[11–14] The arginase activity has also been shown to change in various types of cancer like colorectal cancer, gastric cancer, and non-small cell lung cancer.[11–17] The aim of this study was to examine the arginase activity levels in breast cancer as a marker of this disease.

Materials and Methods

Patients

Fifty healthy females (age (mean ± SD) 48.64 ± 5.17 years, range 29–48 years), 30 female patients with benign breast disease (BBD) (age 53.23 ± 15.74 years, range 26–75 years), 48 female patients with breast cancer (BC) (age 52.67 ± 10.24 years, range 31–68 years), and 7 female patients with breast cancer demonstrating recurrence (bone and lung metastases) after mastec-

tomy (age 69.50 ± 5.78 years, range 60–78 years), were included in this study. With the unity of 50 healthy females and 30 female malignant patients, we also created a new control group (healthy subjects plus benign breast disease group), $n = 80$ (age 45.36 ± 12.04 years, range 26–75 years). Patients with benign disease and breast cancer were hospitalized in the Department of Surgery, and none of them had received either preoperative radiotherapy or chemotherapy. None of the subjects included in these groups had liver disease, and their liver function tests were all within the normal ranges. Since their numbers were too small to be adequately evaluated, two other females with malignant disease having liver metastases (stage IV) were not included in this study. Histologically, of the 30 female patients with benign breast disease, 18 had fibroadenoma, 10 fibrocystic disease, and 2 ductal ectasia. According to the tumor-node-metastasis histological (TNM) classification system,[18] of 48 female patients with breast cancer, 9 had stage I disease (pNo) (age 46.78 ± 9.58 years, range 31–65 years), 18 had stage II disease (pNo, N1a-b) (age 48.83 ± 8.52 years, range 34–62 years), 15 had stage III disease (pN2) (age 58.07 ± 9.07 years, range 38–68 years), and 6 had stage IV disease (M1, bone and lung metastases) (age 59.50 ± 10.17 years, range 46–68 years). As a result, of 48 malignant patients, 27 had stage I + II (early stages) (age 48.15 ± 8.76 years, range 31–65 years) and 21 stage III + IV (advanced stages) (age 58.48 ± 9.16 years, range 38–68 years). As seen from Table 1, 27 malignant patients (6 stage II, 15 stage III, and 6 stage IV) had lymph node metastases. We were able to follow up 25 of 48 malignant patients and we could not follow the remainder because of several reasons such as death and emigration. Routine chest roentgenography, bone scans, and ultrasound were used to detect lung, bone, and liver metastases, respectively.

Blood Sampling

All patients and healthy subjects were entered into the study after obtaining their informed consent. Blood samples were collected by venous puncture and centrifuged, and the sera were frozen at −20°C until the assay day. Blood samples were drawn from all patients both before drug administration and before a mastectomy. Of the 48 female patients with malignant diseases, after a mastectomy blood samples were drawn from 25 patients within the first, second, and third weeks; these patients had all received chemotherapy.

Biochemical Measurements

The serum activities of arginase enzyme were determined according to the method of Chinard which was slightly modified by Porembska and Kedra.[19,20] The enzyme activity measurements were carried out in duplicate. Since erythrocytes contain a relatively high arginase activity, no hemolyzed samples were included in this study.

Statistical Analysis

The data were evaluated with the Student's *t*-test or Mann-Whitney *U*-tests. The Mann-Whitney *U*-test was used to compare the means of the arginase activity levels in evaluating the stages and the presence of lymph node metastases. The Spearman rank correlation method was used to compare the arginase activity and age. The significance level was set at $P < 0.05$. The SPSS for Windows v. 9.0.0 (Chicago, IL, USA) software package was used to analyze the data. The diagnostic sensitivity and specificity of the test were determined by defining a given cutoff point. The following formulas were used for this purpose: sensitivity (%) = [TP/(TP + FN)] × 100, specificity (%) = [TN/(TN + FP)] × 100, and cutoff = mean + 2SD, where TP is the number of true positive values, TN the number of true negative values, FP the number of false positive values, and FN the number of false negative values. Predictive values of positivity and negativity (PPV and NPV) and total accuracy were calculated by using the formulas [TP/(TP + FP)] × 100, [TN/(TN + FN)] × 100, and [(TN + TP)/(TN + TP + FP + FN)] × 100, respectively. The cutoff values (mean + 2SD) were determined to be 13.74 U/l for the healthy group and 17.63 U/l for the benign breast disease group, and 17.68 U/l for the combination of these two groups as a new control group (see Table 2). The SD × 100/mean formula was used to determine the intra-assay (intra-CV) and the interassay coefficient of variations (inter-CV) by using each SD and mean value of these coefficient variations. The receiver-operating characteristics (ROC) curves were performed using the SPSS (Chicago, IL, USA) graph program for Windows v. 9.0.0.

Results

The mean ± SD values of serum arginase activity levels were found to be 9.48 ± 2.13, 11.07 ± 3.28, and 20.35 ± 9.27 U/l in the healthy female controls, in females with benign breast disease, and in females with breast cancer, respectively. The mean value of all subjects with benign breast disease and normal healthy subjects (healthy subjects plus benign breast disease group) was found to be 10.64 ± 3.52 U/l. As seen from Table 1, the serum activity levels of this enzyme were higher in females with breast cancer ($P < 0.001$) than in those of the normal subjects, in the females with benign diseases, or

Table 1. Mean arginase enzyme activity levels in the healthy group, the benign disease group, and the breast cancer group of women

Groups	No. of cases	Mean ± SD age of each group (years)	Mean ± SD of arginase enzyme activity (U/l)
Healthy group	50	48.64 ± 5.17	9.48 ± 2.13
Benign breast disease group	30	53.23 ± 15.74[a]	11.07 ± 3.28[c]
Healthy subjects plus benign breast disease group	80	45.36 ± 12.04	10.64 ± 3.52
Breast cancer group	48	52.67 ± 10.24[b]	20.35 ± 9.27[d]
Stage I	9	46.78 ± 9.58	11.88 ± 1.79
Stage II	18	48.83 ± 8.52	16.47 ± 6.94
Stage III	15	58.07 ± 9.07	23.83 ± 6.40
Stage IV*	6	59.50 ± 10.17	36.00 ± 3.34
Stage I + II	27	49.05 ± 8.97	14.94 ± 6.11[e]
Stage III + IV	21	48.44 ± 11.20	27.31 ± 7.95[f]
0 Ln (or −Ln)	21	57.91 ± 7.41	13.15 ± 4.90[g]
1–3 Ln	9	60.71 ± 9.83	19.80 ± 5.31
4–10 Ln	11	48.15 ± 8.76	24.69 ± 5.51
>10 Ln*	7	58.48 ± 9.16	35.84 ± 3.08
1–3 + 4–10+ >10 Ln (or +Ln)	21	55.48 ± 10.45	25.95 ± 7.92[h]
Remission	25	48.80 ± 8.76	
First week	25		19.60 ± 10.91[i]
Second week	25		13.00 ± 2.54[j]
Third week	25		11.71 ± 1.59[k]
Recurrence	7	69.50 ± 5.78	19.56 ± 10.36[l]

Ln, number of positive lymph nodes
* having bone and lung metastases
[a] vs healthy group; [b] vs healthy, vs benign breast disease, and vs healthy subjects plus benign breast disease groups ($P > 0.05$), Spearman rank correlation
[c] vs healthy group ($P > 0.05$); [d] vs healthy, vs benign breast disease, and vs healthy subjects plus benign breast disease groups ($P < 0.001$), Student's *t*-test
[e] vs healthy and vs healthy subjects plus benign breast disease groups ($P < 0.01$), and vs benign breast disease group ($P > 0.05$); [f] vs healthy, vs benign breast disease, and vs healthy subjects plus benign breast disease groups ($P < 0.01$); [g] vs healthy and vs healthy subjects plus benign breast disease groups ($P < 0.01$), and vs benign breast disease group ($P > 0.05$); [h,i] vs healthy, vs benign breast disease, and vs healthy subjects plus benign breast disease groups ($P < 0.001$); [j] vs healthy, vs benign breast disease, and vs healthy subjects plus benign breast disease groups ($P < 0.01$); [k] vs healthy, vs benign breast disease, and vs healthy subjects plus benign breast disease groups ($P > 0.05$); [l] vs healthy, vs benign breast disease, and vs healthy subjects plus benign breast disease groups ($P < 0.001$), Mann-Whitney *U*-test

Table 2. Diagnostic parameters of the serum arginase enzyme activity levels in patients with breast cancer

Parameters	If the cutoff value is 13.74 U/l[a]	If the cutoff value is 17.63 U/l[b]	If the cutoff value is 17.68 U/l[c]
Sensitivity (%)	66.67	54.17	54.17
Specificity (%)	94.00	96.67	98.75
Postitive predictive value (%)	91.43	96.30	96.30
Negative predictive value (%)	74.60	56.86	78.22
Total accuracy (%)	80.61	65.38	78.91

[a] 13.74 U/l cutoff value was calculated according to the mean value (9.48) of the healthy subjects group and by adding its 2SD (2 × 2.13) value
[b] 17.63 U/l cutoff value was calculated according to the mean value (11.07) of the benign patients group and by adding its 2SD (2 × 3.28) value
[c] 17.68 U/l cutoff value was calculated according to the mean value (10.64) of the healthy subjects plus benign breast disease group and by adding its 2SD (2 × 3.52) value

in the healthy subjects plus benign breast disease group. According to the stages and the number of positive lymph nodes in the females with breast cancer, the mean ± SD levels are given in Table 1. As can be seen, the more advanced the stages and number of positive lymph nodes of breast cancer, the higher the serum level of arginase enzyme activity (see also Figs. 1 and 2). No correlation was found between the arginase activity and age in each group ($P > 0.05$). After a mastectomy, the mean activity levels of this enzyme in 25 patients returned to normal levels within 3 weeks. The activity levels of this enzyme in seven female patients with re-

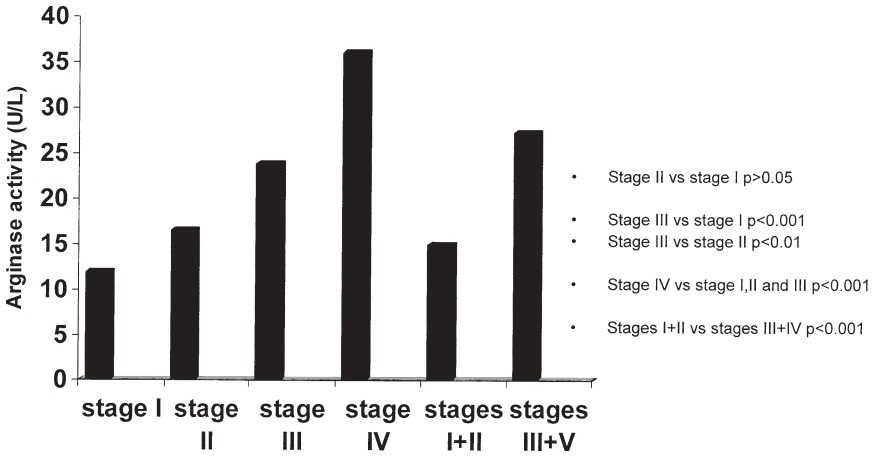

Fig. 1. Bar graph representing the mean activity of the arginase activity (U/l) and the results of the Mann-Whitney *U*-test for the stages of patients with breast cancer

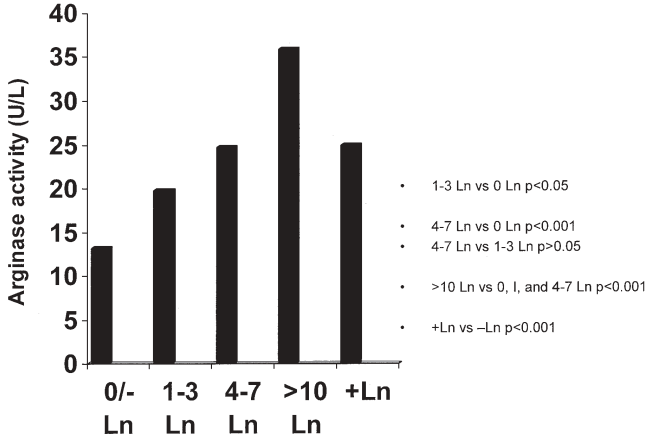

Fig. 2. Bar graph representing the mean activity of the arginase activity (U/l) and the results of the Mann-Whitney *U*-test according to the number of positive lymph nodes of patients with breast cancer. *Ln*, number of positive lymph nodes

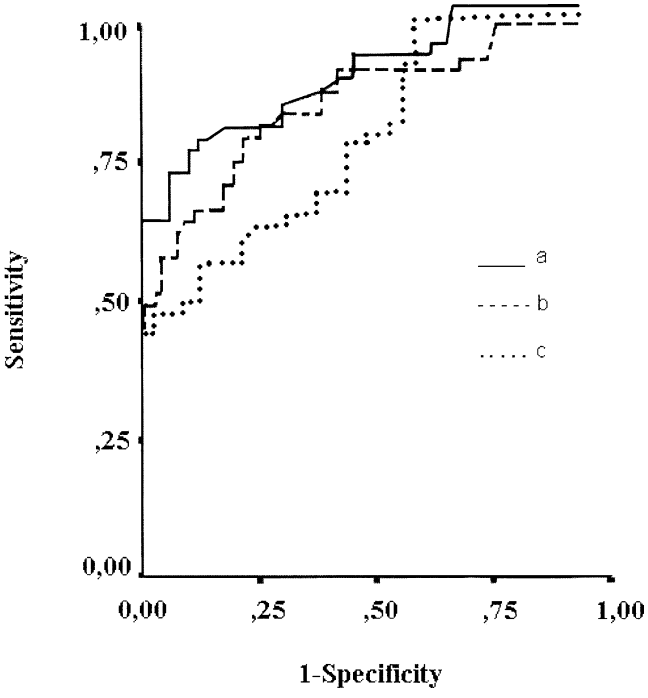

Fig. 3. The receiver-operating characteristics (ROC) curves for the relationship between the diagnostic sensitivity and specificity of arginase enzyme. If the cutoff value is (*a*) 13.74 U/l, (*b*) 17.63 U/l, and (*c*) 17.68 U/l, the ROC curve areas will be 0.887, 0.777, and 0.844, respectively

currence (19.56 ± 10.36) were nearly same with those in the females with breast cancer. Intra-CV and inter-CV were found to be 3.60% and 7.80%, respectively. If healthy subjects are accepted as the control group, the diagnostic sensitivity of serum arginase enzyme activity is higher (66.67%) than those of benign patients and healthy subjects plus benign breast disease control groups (54.17%), while the diagnostic specificity is (94%) lower than the others (96.67% and 98.75%, respectively) (see Tables 2 and 3). The relationship between the diagnostic sensitivity and specificity is illustrated by a ROC curve (see Fig. 3). The arginase enzyme activity areas under the ROC curves of healthy subjects group, benign patients groups, and healthy subjects plus benign breast disease control group were found to be 0.887, 0.773, and 0.844, respectively.

Discussion

Although the function of the arginase enzyme in the liver is the catalysis of arginine hydrolysis to ornithine and urea, in extrahepatic tissues the function of the enzyme is essentially unknown. Normal blood serum contains only trace activity of arginase.[11–17] However, in some pathological conditions, especially in various types of cancer, the elevated activity levels of arginase

Table 3. Sensitivity (%) of serum arginase enzyme activity levels according to the stages and number of positive lymph node metastases (Ln) in patients with breast cancer

Stages and no. of positive lymph node metastases	If the cutoff value is 13.74 U/l sensitivity (%)[a]	If the cutoff value is 17.63 U/l sensitivity (%)[b]	If the cutoff value is 17.68 U/l sensitivity (%)[c]
Stage I	11.12	0	0
Stage II	55.56	44.45	44.45
Stage III	100	86.67	86.67
Stage IV	100	100	100
Early stages (I + II)	40.74	29.63	29.63
Advanced stages (III + IV)	100	90.48	90.48
0 Ln (or −Ln)	28.57	14.29	14.29
1–3 Ln	88.89	55.56	55.56
4–10 Ln	100	100	100
>10 Ln	100	100	100
1–3 + 4–10+ >10 Ln (or +Ln)	96.30	85.18	85.18

Ln, number of positive lymph nodes
[a] 13.74 U/l cutoff value was calculated according to the mean value (9.48) of the healthy subjects group and by adding its 2SD (2 × 2.13) value
[b] 17.63 U/l cutoff value was calculated according to the mean value (11.07) of the benign patients group and by adding its 2SD (2 × 3.28) value
[c] 17.68 U/l cutoff value was calculated according to the mean value (10.64) of the healthy subjects plus benign breast disease group and by adding its 2SD (2 × 3.52) value

enzymes in the serum have been reported in many studies, and it is suggested that arginase enzymes supply ornithine, which is a precursor for the biosynthesis of aliphatic polyamines, to cells.[11–17,21] The polyamines (putrescine, spermidine, and spermine), that are present in all mammalian cells, are essential for cell growth and proliferation and facilitate all the steps of protein synthesis.[21–23] In carcinogenesis, these polyamines and the arginase which supplies these polyamines have also been reported to increase.[21–23] The increase in the arginase enzyme activity appears to participate in increased polyamine formation and may be an important factor in the increased proliferation in carcinogenesis.

In this study, we found elevated arginase enzyme activity levels in the sera of women with breast cancer in comparison with those of healthy subjects, those of patients with benign disease, and those of a combination of these groups as control groups ($P < 0.001$). These results correlate with the findings of previous studies carried out in other cancer types such as gastric, colorectal, large bowel, urogenital, and prostate cancers.[11–16] When the benign group was accepted as the patient group and then was compared with the healthy group as a control, the increases in the arginase activity in the benign group were not statistically significant, which thus indicates that increased activity of this enzyme may be a useful marker in patients with breast cancer. Our results are also in agreement with a previous study carried out by Straus et al.,[22] who previously had shown arginase in human breast tissue by agar gel electrophoresis, but without describing the stages of breast cancer.[24] Breast tumors may cause an increase in arginase enzyme levels. This point has previously been speculated by Wu et al. in a study in which they proposed that in gastric cancer, the increased serum arginase enzyme levels may originate from gastric cancer cells.[12] A larger amount of arginase was found in tumor tissues from colorectal cancer and non-small cell lung cancer than in their adjacent (~10 cm apart) normal tissue, and this finding confirms the release of arginase enzyme from neoplastic cells into the serum.[21,25]

When the benign disease group is considered as a control group, the mean activity of arginase in advanced stage breast cancer and in patients with lymph node-positive (+Ln) disease is also statistically significant at $P < 0.001$, but it is not significant in early stages and in 0 Ln (−Ln). This result suggests that the benign group can also be considered as a control group in patients with advanced stage and a +Ln status of breast cancer.

When the healthy subjects group and the healthy subjects plus benign breast disease group were considered as a new control group, our results for early ($P < 0.01$) and advanced ($P < 0.001$) stages, with positive lymph nodes (+Ln) ($P < 0.001$) or not ($P < 0.01$), also indicated that the increase in the serum arginase activity was related to both the extent of the cancer and to the metastatic cascade. This shows that an increase in the arginase activity levels at the early stages may be a useful marker and may reflect the tumor burden of breast cancer at advanced stages. When patients having positive lymph nodes were compared with those without any positive lymph nodes, the increase in the arginase activity of patients with lymph nodes was significant ($P < 0.001$). This result has also suggested that metastatic tumors may also be an additional origin of an

increased arginase activity level. To prevent the measurement of liver-origin arginase activity, individuals with neither liver metastasis nor liver disease were included in this study.

In the serum samples, obtained within the first, second, and third weeks after a mastectomy, the activity levels of this enzyme were observed to decrease significantly, thus indicating that this enzyme activity may be useful for monitoring the therapy. In addition to the 48 patients with breast cancer, another seven female patients with breast cancer having recurrence also had demonstrated a high arginase activity, which may be explained by the incomplete removal of the primary tumors. This increase also suggested that the increased arginase activity levels may be a useful marker in monitoring the recurrence of the disease. Recently, many studies have suggested serum arginase to be an immune suppressive factor, but his role of the enzyme in tumors is still not clear.[11,20] The cutoff values (mean + 2SD) of this test were calculated according to the mean + 2SD of the healthy subjects group, benign breast disease group, and healthy subjects plus benign breast disease group, which were 13.74, 17.63, and 17.68 U/l, respectively. An ideal tumor marker should possess high sensitivity (the marker should be detectable at the very early stage of the tumor) and specificity (the marker should not be detectable in healthy subjects). However, up to now, none of the tumor markers have fulfilled the criteria of 100% sensitivity and 100% specificity. As seen from the Table 2, if the cutoff value is accepted as 17.63 U/l or 17.68 U/l, the sensitivity is lower (54.17%) in comparison with the 13.74 U/l cutoff value (66.67%). However, the specificity of this test increases with an increasing cutoff value. As a result, these three cutoff values were considered to be useful for the diagnostic use of arginase enzymes as a tumor marker. The positive and negative predictive values for the 17.68 U/l cutoff value are the highest, but the total per cent accuracy of the 13.74 U/l cutoff value was the highest. Both the specificity and sensitivity of this enzyme activity also increases as the stage increase and as the number of lymph nodes increase (see Table 3). The area under the ROC curve represents the clinical usefulness of tumor markers: the larger the area under the ROC curve, the higher the usefulness of the tumor marker. In this study, according to the cutoff values of healthy subjects, benign patients, and healthy subjects plus benign breast disease group as control groups, the ROC curve areas are 0.887, 0.773, and 0.844, respectively (see Fig. 3). Although the first value is the highest, this statistical result suggests that each group (healthy subjects, benign patients, or healthy subjects plus benign breast disease groups) has a similar performance in comparison with each other, and thus each of them may be used as a control group.

In conclusion, the high serum activity levels of arginase enzyme in breast cancer may originate from breast tumors. In other words, the higher the activity levels in the serum, the higher the stages of the disease. As a result, the arginase enzyme activity levels could be used to improve the clinical assessment of breast cancer and might also be useful as a biological marker associated with the malignant stage and the progression of the tumor. However, according to the localization of tumors and metastases, especially regarding liver metastases, further studies in more patients are needed. Whether or not a correlation exists between the arginase activity and other markers remains to be elucidated. After the amino acid sequences of extrahepatic isoenzymes of arginase have been characterized or specific antibodies to each of them developed, using either the quantitative reverse transcriptase-polymerase chain reaction method[26] or immunological reactions, the increases in the arginase enzyme levels are expected to be more clearly elucidated.

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