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Superoxide-induced deimination of arginine in hematopoietic cells

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Murine bone marrow cells can produce citrulline directly from L-arginine without intermediate ornithine. An L-arginine-dependent biochemical pathway synthesizing L-citrulline and nitrate, coupled to an effector mechanism has also been recently demonstrated in murine cytotoxic activated macrophages. We show herein that L-citrulline synthesis in murine bone marrow cells can be induced by the generation of superoxide. It can take place in an arginine-free medium, suggesting the implication of a superoxide-dependent peptidyl arginine deiminase.

L-Citrulline synthesis; Arginine deiminase; Superoxide; (Mixed leukocyte culture)

L-Citrulline synthesis by murine spleen and bone marrow cells incubated with secondary mixed leukocyte culture (MLC) supernatant has been described in [1]. More recently [2], this production has also been demonstrated in cytotoxic activated macrophages. In both cases citrulline is generated by arginine deimination. Since arginine deiminase has not been described in mammalian cells before [3], we have studied the mechanism involved in citrulline synthesis.

L-Citrulline accumulation in the medium was measured after incubation of cells or in enzymatic assay medium by a colorimetric reaction that detects carbamido compounds (non-urea diacetylmonoxime-reacting compounds). Urea was removed from samples prior to assay by incubation with urease (6.4 units/ml) at 37°C for 1 h. The identity of the diacetylmonoxime-reacting compound with citrulline has been established by gas chromatographic mass-spectrometric analysis [1]. The citrulline production was low or undetectable when bone marrow cells were incubated alone and increased, when dialyzed secondary MLC supernatant was added to the culture medium (table 1). The same results were obtained with a partially purified secondary MLC supernatant (active fractions eluting at a molecular mass of 150 kDa after Ultrogel AcA 34 gel-exclusion chromatography). A small increase of the citrulline production was also observed with spleen cells (51.5 ± 2.5 nmol/10^7 cells against 121.2 ± 8.5 nmol/10^7 bone marrow cells after a 48 h incubation), but not with thymus, lymph node cells or resident peritoneal macrophages.

The involvement of ornithine transcarbamylase in citrulline production is ruled out since: (i) L-[*guanido*-^{14}C]arginine is transformed by bone marrow cells into L-[*ureido*-^{14}C]citrulline in response to MLC-derived factor(s) and (ii) bone marrow cells from spf mice, deficient in ornithine transcarbamylase [4], still respond to the secondary MLC supernatant by citrulline synthesis. Arginine deiminase, an enzyme producing citrulline by deimination of arginine in prokaryotes, was assayed on lysed bone marrow cells according to Schimke's method [5] but no citrulline production was obtained in these conditions. Peptidylarginine deiminase which can transform arginine residues of proteins into citrulline residues was also assayed by the method described by Rothnagel and Rogers

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Table 1

Citrulline production by bone marrow cells

Incubation time (h)	Citrulline production (nmol/10^7 cells) by bone marrow cells incubated with	
	Culture medium	Secondary MLC supernatant (50% dilution)
12	undetectable	5.5 ± 0.9
24	undetectable	38.6 ± 1.6
48	2.3 ± 0.5	121.0 ± 8.5
72	6.5 ± 1.6	215.6 ± 20.9
96	9.3 ± 2.3	235.9 ± 16.3

10^7 bone marrow cells obtained from C57BL/6 mice were suspended in an RPMI low endotoxin culture medium (Gibco) without serum, supplemented with 1% sodium pyruvate, 1% L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin. The absence of bacterial or mycoplasma contamination in cell cultures has been regularly verified. The mixed leukocyte culture (secondary MLC) supernatant was established between skin allograft donor [DBA/2 (H-2^d) mice] and recipient [C57Bl6 (H-2^b) mice] spleen cells as described [1]. After 48 h of incubation, supernatants were collected, dialyzed against distilled water and lyophilized. The residues were then redissolved in cultured medium, sterilized and tested at a 50% dilution

[6] using 40 mM *N*-benzoyl arginine as substrate. Citrulline production was not observed. However, when a system generating superoxide (4 mM xanthine and 0.005 IU xanthine oxidase) was added to bone marrow cell lysates, citrulline production took place, whether arginine was added or not (fig.1). Citrulline production in bone marrow cells is thus catalyzed by a superoxide-dependent peptidylarginine deiminase. This enzyme is different from the one previously described since in our experiments citrulline synthesis is sustained by Ca^{2+}-free dialyzed bone marrow cell lysates, whereas peptidylarginine deiminase has an absolute requirement for Ca^{2+} [6].

Moreover, the citrulline residues resulting from the action of this new enzyme must be released from the proteins probably by proteases, present in bone marrow cell lysates [7]. It seems that secondary MLC supernatant acts upon citrulline synthesis by increasing the superoxide production required for the deimination of arginine.

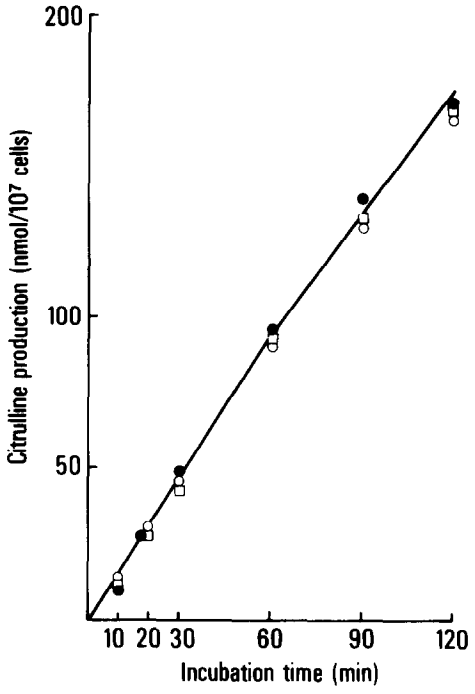

Fig.1. Citrulline production in bone marrow cell lysates in the presence of 40 mM xanthine and 0.005 IU xanthine oxidase. Bone marrow cells were lysed in 10 mM phosphate buffer, pH 7.0, by repeated freeze-thawing cycles. Lysates from 5×10^6 bone marrow cells were incubated with 4 mM arginine in a final volume of 200 μl phosphate buffer. The addition of xanthine/xanthine oxidase greatly enhances the transformation of arginine into citrulline (●). The reaction proceeds in arginine-free medium (□) as well as in bone marrow cell lysates dialyzed against phosphate buffer (○). No citrulline production was observed in the absence of xanthine or xanthine oxidase.

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