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Dedifferentiation Correlates with the Expression of Lysosomal Acid Phosphatase in the Limb Regenerates of Mexican Axolotl

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멕시코산 엑소로틀 다리 재생조직의 탈분화와 리소솜 산성탈인산화효소의 발현

서광석 · 박숙경 · 주봉건 · 전상학* · 김원선

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ABSTRACT : The lysosomal acid hydrolases including lysosomal acid phosphatase (LAP) are believed to play an important role in intracellular and extracellular degradation. LAP was reported to increase its activity in dedifferentiation stage during urodele limb regeneration. In the present study, LAP localization in the Mexican axolotl (*Ambystoma mexicanum*) limb regenerates was investigated by immunohistochemistry. LAP immunoreactivity with monoclonal antibody against Korean salamander (*Hynobius leechii*) LAP was observed mainly in the wound epidermis, blastema cells, muscle, and cartilage which were under dedifferentiation process in axolotl limb regenerates. Moreover, LAP immunoreactivity increased gradually during the early phase of limb regeneration and reached the peak level at dedifferentiation stage. However, as redifferentiation begins, LAP immunoreactivity decreased slowly to the basal level. Retinoic acid (RA) which is known to induce skeleton pattern duplication in regenerating urodele limb appears to enhance LAP immunoreactivity. In the RA-treated limb regenerates, LAP immunoreactivity was higher than the normal regenerates. In addition, the LAP expression period was more extended in the RA treated regenerates than in the normal regenerates. These results suggest that RA is involved in the extension of dedifferentiation state in RA-treated limb regenerate.

Key words: Lysosomal acid phosphatase, Regeneration, Dedifferentiation, Retinoic acid, Axolotl.

요 약 : 리소솜 acid phosphatase (LAP)를 포함하는 리소솜 산성 가수 분해 효소는 세포 내,외 성분의 분해에 중요한 기능을 수행하며, 유미 양서류의 다리 재생 과정에서 LAP의 효소 활성도는 탈분화시기에 특이적으로 증가하는 것으로 알려져 있다. 본 연구에서는 멕시코산 도롱뇽의 일종인 axolotl (*Ambystoma mexicanum*)의 다리 재생 과정에서 정상적인 다리 재생 조직과 retinoic acid (RA)가 처리된 다리 재생 조직에서 LAP의 분포 및 발현을 면역 화학적 방법으로 조사하였다. Axolotl의 다리 재생 조직에서 한국산 도롱뇽 (*Hynobius leechii*)의 LAP에 대한 단일 항체를 이용한 면역 반응도는 주로 탈분화시기에 있는 wound epidermis와 미분화된 중배엽성 세포인 blastema 세포, 탈분화 상태의 근육과 연골 부위 세포에서 나타나는 것으로 조사되었다. 또한 LAP의 면역 반응도는 재생 단계의 초기에 점차 증가하여 탈분화시기 동안 최대치에 도달하였으며, 재분화가 시작되면서 basal level까지 서서히 감소하는 것으로 나타났다. 유미 양서류의 다리 재생 과정에서 골격 패턴의 복제를 유발시키는 것으로 알려져 있는 RA 처리는 LAP의 면역 반응도를 강화시키는 것으로 나타났다. 즉, RA가 처리된 다리 재생 조직에서 LAP의 면역 반응도는 정상적인 다리 재생 조직에서 보다 강하게 나타났으며, 면역 반응도가 나타나는 시기 또한 연장되는 것으로 조사되었다. 이와 같은 결과는 다리 재생 조직에서 RA 처리가 탈분화시기의 연장에 관여한다는 것을 시사하고 있다.

INTRODUCTION

In the study of pattern formation, regenerating limbs of salamanders have been used frequently as a prominent model system due to their remarkable regenerative power (reviewed in Stocum, 1979).

Regeneration process of salamander limbs can be divided into four phases (Stocum, 1979). The first phase is an inflammation and cell migration phase called 'wound hea-

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ling'. When urodele limbs are amputated, inflammatory response occurs immediately, with the retraction of soft tissues and the formation of plasma clots at the wound surface. Simultaneously, the epidermal cells in the remaining stump start to migrate to wound surface and proliferate to cover the injured surface. Moderate tissue necrosis, migration of neutrophils and macrophages to the injured site also take place at this phase (Slack, 1982). The second phase is the dedifferentiation stage. In this stage, cells in the stump tissue lose their differentiated features. The third phase is blastema formation. Dedifferentiated cells from stump proliferate to form blastema which have the characteristics of embryonic mesenchymal cells. The final step of regeneration is redifferentiation of blastema by which restoration of missing part is accomplished. During this process, embryonic cell-like blastema cells differentiate into muscle cells, chondrocytes, osteocytes and neuronal cells, etc.

In the limb regeneration, only distal part of amputation level can be regenerated. This phenomenon called 'rule of distal transformation' means that 'positional value' in the blastema cells can be only distalized to form the distal structure from amputation level (Rose, 1970; Wolpert, 1989). 'Positional value' is thought to be imprinted on the cell as a result of interpretation of positional information at the early stage of limb development (Wolpert, 1969, 1989).

In the regeneration process, dedifferentiation is a critical event since the supply of blastema cells is feasible by this process. Therefore, if this step is perturbed, abnormal type of regeneration will occur. The dedifferentiation poses two interesting questions. The one is how the blastema cells form from stump tissue since blastema cells restore its previous developmental potency due to the dedifferentiation process. The second question is how the positional values of dedifferentiated cells are retained and what the nature of positional value is. Especially, the answer for the second question appears to be directly related to understanding the fundamental mechanism of pattern formation. In the study of pattern formation, retinoic acid (RA) has been used frequently since it evokes various types of pattern alterations (Maden, 1982; Niazi et al., 1985). For example, excessive RA causes teratogenicity in the developing systems such as eye, heart, brain and palate in vertebrate embryos (Altaba, 1991; Creton et al., 1995). In mouse embryos, excessive RA causes craniofacial abnormalities, deletion of digit structure and reduction of limbs (Kochhar, 1973; Sulik et al., 1988). In *Xenopus* embryo, excessive RA induces reduction of hindbrain and malformation of eyes (Altaba, 1991; Creton et al., 1995). In the regenerating limbs of amphibians, RA causes pattern duplication in dose, regenerating stage, and amputation level dependent manners (Kim and Stocum, 1986; Lee and Kim, 1990; Ju and Kim, 1994).

In the dedifferentiation and blastema formation process, the existing extracellular matrix (ECM) in the stump must be degraded to be replaced by embryonic ECM (Stocum, 1995). In this process, many kind of proteases such as metalloproteinases and lysosomal acid hydrolase are involved in degradation (Schmidt, 1968; Matrisian, 1990). In the regressing tail of metamorphosing *Xenopus* tadpole, acid phosphatase, cathepsin and collagenases are known to degrade ECM (Weber, 1963; Eisen and Gross, 1965; Filburn, 1973).

Recently, the activities of lysosomal acid phosphatase (LAP) and various kind of proteinases were examined in the limb regenerates of Korean salamander, *Hynobius leechii* (Ju and Kim, 1994; Lee and Kim, 1996; Park, 1995). The activities of LAP, trypsin-like and chymotrypsin-like proteinases, and various types of matrix metalloproteinases reach the maximum level at dedifferentiation stage. Moreover, it was found that RA treatment led to the augmentation and prolongation of activities of these enzymes. Recently, we prepared monoclonal antibody (mAb) against LAP (53 kDa) of *H. leechii* and it was shown to have strong cross-reactivity to LAP of Mexican axolotl (*Ambystoma mexicanum*) and *Xenopus laevis* (Ju et al., 1996).

In the present study, the profile of LAP expression in the limb regenerates of *Ambystoma mexicanum* were surveyed immunohistologically and the effects of RA on LAP expression were examined at various stages of regeneration to understand the role of LAP in the process of dedifferentiation.

MATERIALS AND METHODS

1. Experimental animals and RA injection

Mexican axolotl, *Ambystoma mexicanum*, was used in this study. The eggs of Mexican axolotl were obtained by mating stock animals. Newly hatched larvae were kept in dechlorinated tap water and were fed with freshly hatched brineshrimp or finely chopped bovine heart. Axolotl larvae were reared in grid container and paper cups in separation to prevent cannibalism. At the time of amputation, the larvae were 50~60 mm in length and 3~4 g in body weight. Before amputation, animals were anesthetized in 0.02% benzocaine (Sigma) solution. The forelimbs were amputated at distal stylopodial level (upper arm) and any protruding cartilages were trimmed to get a flat amputation surface.

2. Preparation and administration of retinoic acid (RA)

Retinoic acid (all trans, type XX, Sigma) was dissolved in dimethyl sulfoxide (DMSO, Sigma) at the concentration of 50 mg/ml under dim light to prevent photo-isomerization. Before RA injection, axolotls were weighted on a top-loading balance to determine the total amount of RA to be injected. Animals were anesthetized in 0.02% benzocaine dissolved in filtered tap water and RA was injected intraperitoneally using a microliter syringe (Hamilton Co., Reno, NV). Each animal was injected with the dose of 150 μg of RA/g body weight at 4 days after amputation (Kim and Stocum, 1986).

3. Tissue preparation and immunohistology

For the immunohistological study of LAP in regenerating limbs, regenerates were collected at 2 days interval from 0 day (immediately after amputation) to 22 days after amputation in normal regeneration groups and from 2 days after RA injection to 24 days after RA injection in RA treated groups. The collected regenerates were embedded with O. C. T. compound (Miles inc.) and kept −70℃ until use. Larval limb regenerates of axolotl were cryosectioned serially at 10 m thickness and tissue sections were mounted on gelatin-coated slides.

The tissue sections were fixed in 4% paraformaldehyde for 15 minutes and rinsed with tris-buffered saline (TBS; 5 mM $CaCl_2$, 5 mM KCl, 10.5 mM $MgCl_2$, 137 mM NaCl, 1.4 mM Na_2HPO_4, 25 mM Tris base). Fixed tissue sections were permeabilized with 0.3% Triton X-100 in TBS for 15 minutes and then immersed in 0.5% bovine serum albumin (BSA) in TBS for 15 minutes. Since a monoclonal antibody against *Hynobius* LAP (mAb HlAcp 10) showed the strongest immunoreactivity in the limb regenerate of Mexican axolotl, this mAb was used as a probe to analyze the localization and expression of LAP during axolotl limb regeneration. Tissue sections were incubated with monoclonal antibody against *Hynobius* LAP at room temperature for 3 hours in a moisture chamber. After washing thoroughly with TBS twice for 3 minutes, sections were treated with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (Sigma, diluted 1:20 in TBS/BSA) for 3 hours in the dark condition. After thorough washing with TBS, the sections were mounted in Gelvatol. The mounted sections were viewed and photographed with Kodak TMAX 3200 under fluorescence filter (I2/3, Leitz). As a negative control, tissue sections were treated with FITC-conjugated goat anti-mouse IgG without prior incubation with mAb HlAcp 10.

RESULTS

1. Expression profile of LAP in normal limb regenerates

To confirm that mAb HlAcp 10 has a specific immunoreactivity against axolotl LAP, immunohistochemistry was performed. In the axolotl limb regenerates, strong LAP immunoreactivity was noted with mAb HlAcp10 whereas only background level of signal was observed with secondary antibody alone (Fig. 1). In the normal limb regenerates, immunoreactivity was detected in the various tissues including wound epidermis, dermis, muscle and cartilage. As shown in Fig. 2, expression profiles of LAP immunoreactivity during normal limb regeneration were dependent on regeneration stage. Immediately after amputation, LAP immunoreactivity was strong in epidermis (Fig. 2A). At wound healing stage (Fig. 2B), LAP immunoreactivity was strong in wound epidermis, muscle, cartilage and perichon-

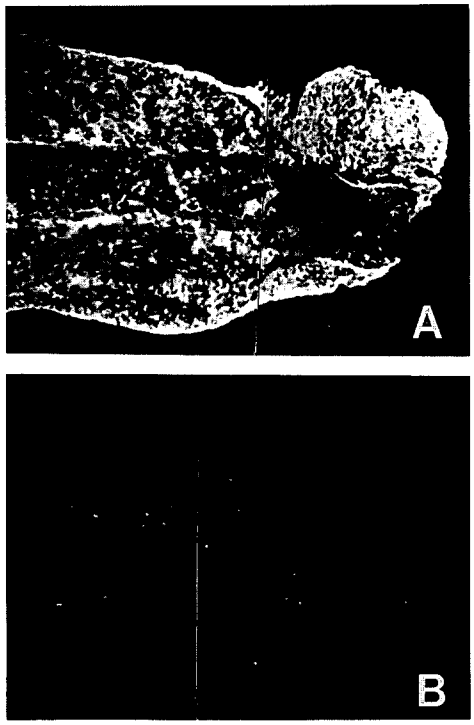

Fig. 1. Crossreactivity of the HlAcp10 to axolotl limb regenerate.
A: LAP expression in the RA-treated limb regenerate at 12 days after RA injection surveyed with H1Acp10.
B: Negative control of RA treated limb regenerate at 12 days after RA injection. Background level of immunoreactivity resulted from the staining with secondary antibody alone.

drium. As regeneration proceeded, intense LAP immunoreactivity was noted in the wound epidermis (Fig. 2C and 2D). High level of LAP immunoreactivity was detected in the wound epidermis and blastema cells, and the peak level was attained at dedifferentiation and early bud stages when the thickened wound epidermis was still prominent at the distal tip of the regenerating limbs (8-10 days after amputation; Figs. 2E-2G). By 16 days after amputation, histolysis at the tip of the stump cartilage was dwindled and LAP immunoreactivity was also low (Fig. 2H). Thereafter, LAP immunoreactivity became even lower in both stump tissue and the regenerating blastema (Figs. 2H-2L).

Table 1 summarizes relative LAP immunoreactivities in the various stump tissues during normal limb regeneration. In the wound epidermis, the LAP immunoreactivity gradually increases until 10 days after amputation and decreases thereafter. In cartilages, LAP immunoreactivity increases until 12 days after amputation and slowly decreases. The immunoreactivity in the cartilage and its surrounding tissue showed some spatial difference depending on regeneration stages. Perichondrium exhibited relatively strong LAP immunoreactivity throughout dedifferentiation stage and early blastema formation stage, but LAP immunoreactivity in perichondrium was not significant once the degradation of cartilage became fading. In chondrocytes, LAP immunoreactivity was relatively strong during the whole period of limb regeneration. Distal tip of cartilage exhibited strong immunoreactivity and its level was maximum when cartilage began to be degraded. In blastema mesenchyme matrix, LAP immunoreactivity showed peak level of signal at 10 days after amputation and it gradually decreased thereafter. In dermis, the immunoreactivity was low but the temporal profile of immunoreactivity was very similar to that in wound epidermis.

2. Expression profile of LAP in RA-treated limb regenerates

Expression profile of LAP in the RA-treated regenerating limbs of axolotl is shown in Fig. 3. Generally, RA treatment induced increased immunoreactivity concomitantly with the prolonged and enhanced dedifferentiation state. The LAP immunoreactivity increased dramatically from 2 days after RA injection (6 days after amputation) to 24 days after RA injection (28 days after amputation). The spatial expression pattern of LAP in RA-treated regenerating limbs was similar to that of normal regenerates. The signal of LAP immunoreactivity was found to be present mainly in wound epidermis, muscle, cartilage and blastema mesenchymal matrix. From 2 days after RA injection (6 days after amputation) LAP immunoreactivities gradually increased and reached maximum level at 12 days after RA injection (16 days after amputation).

Wound epidermis showed strong LAP immunoreactivity until redifferentiation started. Well developed thickened wound epidermis was prominent from 4 days after RA injection (Fig. 3B), and its LAP immunoreactivity reached a maximum level at 12 days after RA injection. Distal tip of cartilage showed strong immunoreactivity during dedifferen-

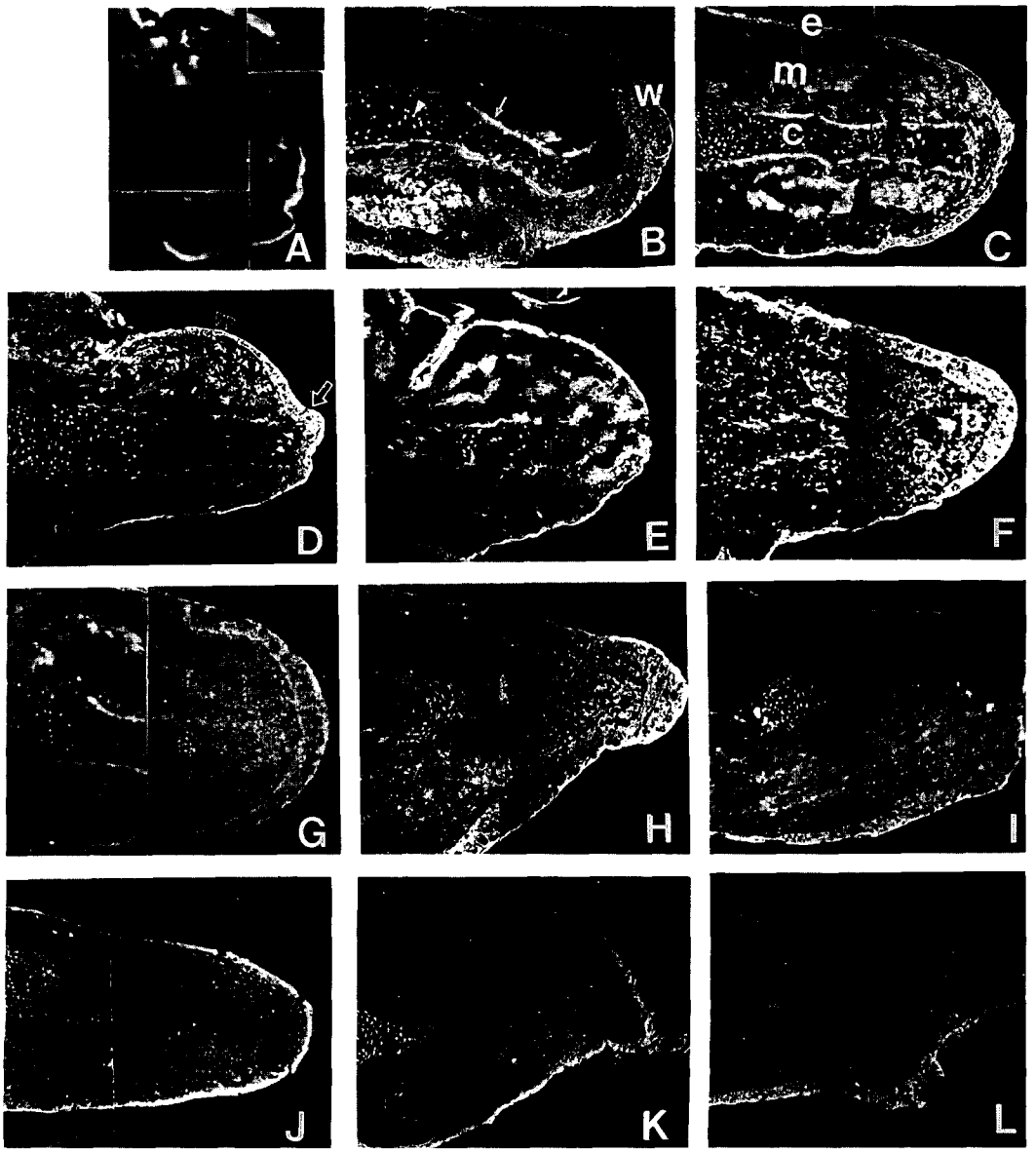

Fig. 2. The expression profile of lysosomal acid phosphatase (LAP) in the normal limb regenerates of Mexican axolotl (*Ambystoma mexicanum*). Magnification, × 60. The LAP expression was examined immunohistochemically using mAb H1Acp10 to *Hynobius* LAP.

A. Immediately after amputation.

B. 2 days after amputation. Note the intense immunoreactivity in the wound epidermis. Also some LAP immunoreactivity in the perichondrium of the stump cartilage is observable. Arrow; perichondrium, Arrow head; chondrocytes.

C. 4 days after amputation. Intense LAP immunoreactivity is present in the dedifferentiating muscle (m) and cartilage (c). e; stump epidermis.

D. 6 days after amputation. Thickened wound epidermis (open arrow) shows much stronger immunoreactivity than the stump epidermis (open arrow head).

E. 8 days after amputation. Distal tip of cartilage is very reactive with mAb H1Acp10.

F. 10 days after amputation. Immunoreactive blastema cells are present beneath the wound epidermis. b; blastema cells.

G. 12 days after amputation. Growing blastema is filled with LAP positive cells under the wound epidermis.

H. 14 days after amputation. LAP immunoreactivity starts to fade.

I. 16 days after amputation. Declining LAP immunoreactivity with onset of redifferentiation of blastema cells.

J. 18 days after amputation.

K. 20 days after amputation.

L. 22 days after amputation. Note the basal level of LAP immunoreactivity in J-L.

Table 1. Relative LAP immunoreactivity¶ during normal limb regeneration

Tissue /cells examined	Days after amputation											
	0	2	4	6	8	10	12	14	16	18	20	22
WE[1]	N	++	+++	+++	+++	+++	++	++	+	++	+	+
Chondrocyte	+	++	++	++	++	++	++	++	N	N	N	N
PC[2]	+	+++	+++	+++	+++	+++	+++	++	+	N	N	N
Distal tip of cartilage	N	+	++	++	+++	+++	++	+	N	N	N	N
Muscle	++	+++	++	++	++	++	++	+	+	N	N	N
MM[3]	N	N	N	N	N	+++	++	++	+	+	+	+
Dermis	+	+	++	++	++	+++	++	++	+	+	+	+

¶ Assessed by examining immunoreactivity on sections on a scale of 1(+) to 3(+++) (+++; highly reactive, ++; moderately reactive, +; slightly reactive).
[1] Wound epidermis [2] Perichondrium [3] Mesenchymal matrix
N : Data not available at the specified days after amputation.

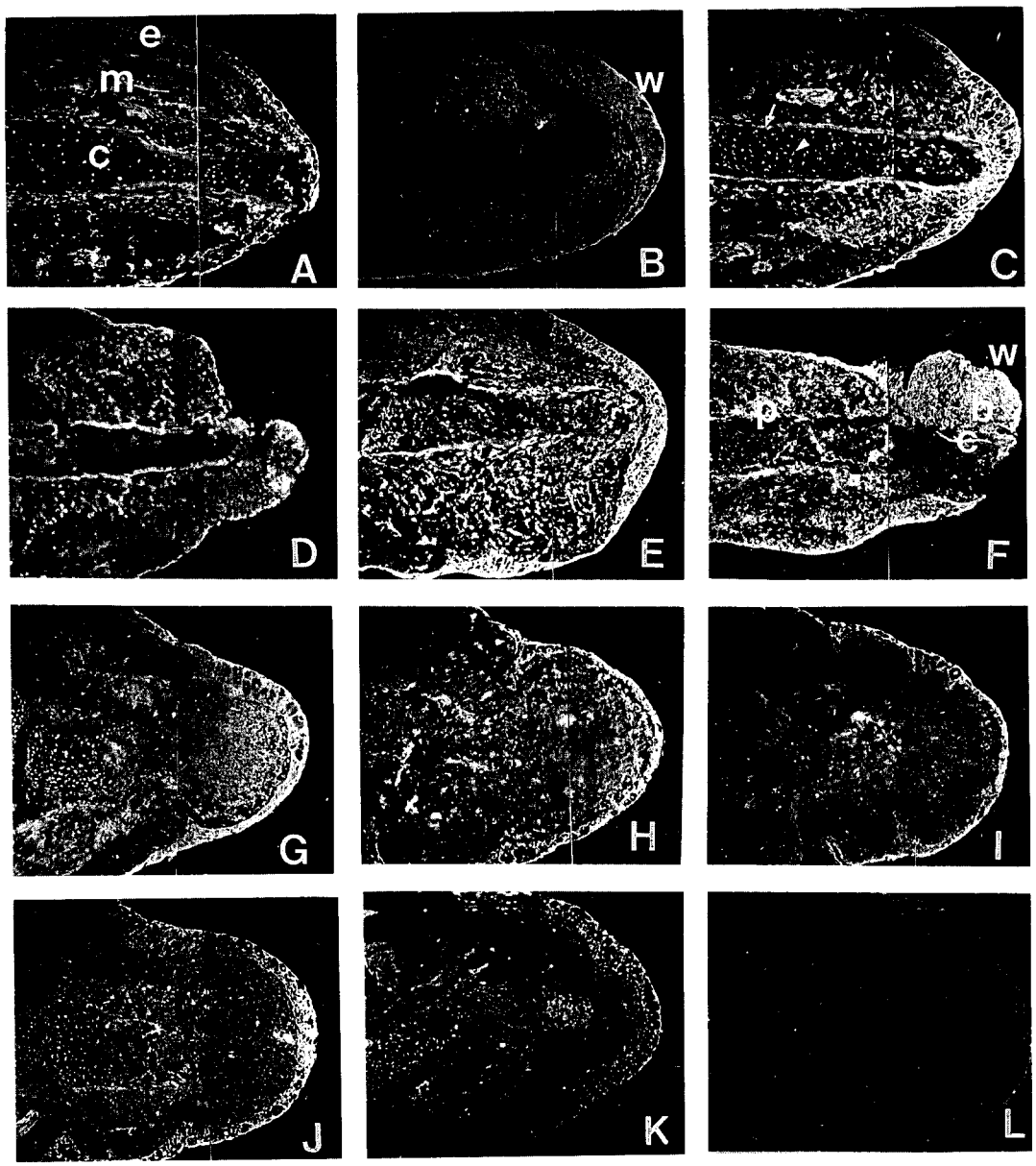

Fig. 3. The expression profile of lysosomal acid phosphatase (LAP) in the RA-treated limb regenerates of Mexican axolotl.
Magnification, × 60. The LAP expression was detected immunohistochemically using mAb H1Acp10 to *Hynobius* LAP.

Table 2. Relative LAP immunoreactivity¶ during RA treated limb regeneration

Tissue /cells examined	Days after amputation											
	6	8	10	12	14	16	18	20	22	24	26	28
WE[1]	++	+++	+++	+++	+++	+++	+++	+++	++	+++	+	+
Chondrocyte	++	A	++	++	++	++	N	N	N	N	N	N
PC[2]	++	A	++	++	++	+++	N	N	N	N	N	N
Distal tip of cartilage	+	++	+++	+++	+++	+++	N	N	N	N	N	N
Muscle	++	++	+++	+++	+++	+++	++	++	N	N	N	N
MM[3]	N	N	++	++	++	+++	+++	+++	++	+	+	+
Dermis	++	+	++	++	+++	+++	++	++	+	+	+	+

¶ Assessed by examining immunoreactivity on sections on a scale of 1(+) to 3(+++)
(+++; highly reactive, ++; moderately reactive, +; slightly reactive).
[1] Wound epidermis [2] Perichondrium [3] Mesenchymal matrix
A: Since cartilage was lost when limb tissue was sectioned, LAP immunoreactivity was not detected.
N: Data not available at the specified days after amputation.

tiation stage (Figs. 3C-3F). However, in the dermis, mAb HlAcp 10 reactivity was not so strong as that in the wound epidermis. As shown in Fig. 3G and 3H, blastema mesenchymal matrix showed increased cell number and strong LAP immunoreactivity at 14~16 days after RA injection. Compared to normal limb regenerates, LAP immunoreactivity in blastema was more intense and blastemal cell density was higher than the control.

Table 2 summarizes the profiles of LAP immunoreactivity in RA- treated limb regenerates. Wound epidermis showed maximum level of immunoreactivity at 16 days after amputation and the level declined thereafter. Perichondrium showed strong LAP immunoreactivity throughout dedifferentiation stage (Figs. 3C-3F). These expression profiles of LAP immunoreactivity was similar to those of normal limb regenerates. With the onset of muscle redifferentiation, LAP immunoreactivity started to decline (Figs. 3I-3L). Dermis was less immunoreactive to mAb against LAP but showed relatively strong immunoreactivity from 8 days after RA injection to 16 days after RA injection (Figs. 3D-3H).

DISCUSSION

The lysosome and its acid hydrolase are believed to play an important role in the process of intracellular and extracellular digestion (Holtzman, 1989). In the urodele limb regeneration, various differentiated tissues are degraded before blastema formation. The activities of lysosomal enzymes, such as lysosomal acid phosphatase, cathepsin and collagenase, are known to increase during the early phase of urodele limb regeneration (Stocum, 1995). Among these

A. 2 days after RA injection. m; muscle, c; cartilage, e; epidermis.
B. 4 days after RA injection. Note the thickened wound epidermis at the distal tip. w; wound epidermis.
C. 6 days after RA injection. Intense immunoreactivity is observable under the wound epidermis. Perichondrium and distal tip of cartilage show strong LAP immunoreactivity. arrow; perichondrium, arrowhead; chondrocyte.
D. 8 days after RA injection. An extensive tissue degradation is under way in the distal region with strong LAP immunoreactivity.
E. 10 days after RA injection.
F. 12 days after RA injection. Note the intense LAP immunoreactivity in the ectopic blastema. wound epidermis (w), distal tip of cartilage (c), perichondrium(p) and blastema (b).
G. 14 days after RA injection. A compact ball of blastema cells shows high level of immunoreactivity.
H. 16 days after RA injection. LAP immunoreactivity begin to decrease.
I. 18 days after RA injection. Still, wound epidermis shows strong LAP immunoreactivity.
J. 20 days after RA injection.
K. 22 days after RA injection.
L. 24 days after RA injection. LAP immunoreactivity shows basal level of signal.

lysosomal acid hydrolase, lysosomal acid phosphatase (L-AP) has been reported as a marker representing lysosomal enzyme activities (Rasch and Gawlik, 1964).

In the present study, the signal of LAP expression was detected in the wound epidermis, cartilage, muscle, dermis, and blastema mesenchymal matrix of the axolotl limb regenerates. At the early phase of regeneration (from 2 days to 4 days after amputation), LAP immunoreactivity was detected in wound epidermis, perichondrium, muscle and distal tip of cartilage with strong signal. Since macrophages and other phagocytes that contain LAP are known to migrate into the trauma zone close to the wound surface during urodele limb regeneration, wound epidermis and distal tip of cartilage are supposed to exhibit strong LAP immunoreactivity (Slack, 1982; Adams and Hamilton, 1992).

As dedifferentiation proceeded, wound epidermis showed intense immunoreactivities as muscle and cartilage did. The LAP immunoreactivity dramatically increased to the peak level during dediffrenetiation stage (4-10 days after amputation) and slowly decreased thereafter. During the dedifferentiaion stage, distal tip of cartilages, perichondrium, wound epidermis and muscle showed high level of LAP immunoreactivity. Moderate level of LAP immunoreactivity of chondrocytes has been maintained throughout regeneration. However, as redifferentiation began, distal tip of cartilages, perichondrium, wound epidermis and muscle showed decreased level of LAP immunoreactivity. Thus, it appears that LAP activity and its expression are closely related to the state of tissue dedifferentiation. Generally, lysosomal acid hydrolases are thought to be required during the dedifferentiation of the stump tissues to catalyze the breakdown of extracellular matrix (ECM) (Stocum, 1995).

Previously, we have found that LAP activity (based on the enzyme assay) was dramatically increased at dedifferentiation stage in *Hynobius* limb regeneration (Ju and Kim, 1994). Also, immunohistological study of LAP in *Hynobius* showed that LAP immunoreactivity was somewhat intense in epidermis while its signal was weak in skeletal muscle and cartilage at wound healing stage. But during dedifferentiation stage, the intensity of immunoreactivity became strong in the wound epidermis, muscle, perichondrium and tips of cartilage. These results are in good agreement with the enhanced LAP activity at dedifferentiation stage (Ju et al., 1996). The activity and localization of active LAP in the limb regenerates have also been reported in other urodele species. In the limb regenerates of *Nothopthalmus viridescens*, LAP activity was detected in epidermis, dermal fibroblasts, subepidermal glands, skeletal muscles and nerve tissues (Miller and Wolfe, 1968). Weiss and Rosenbaum (1968) reported that the strongest LAP activity was detected in macrophages and leukocytes migrated to the distal regions of the regenerating limb in spotted salamander (*Ambystoma maculatum*). These results are similar to the activity staining data of *Hynobius* (Ju and Kim, unpublished data). In fact, after amputation, macrophages migrate to the wound epidermis and subepithelial space in the early phase of regeneration (Schmidt, 1968). Macrophages are known to have many kinds of degradative enzymes such as matrix metalloproteinases for collagens and gelatin, cathepsin, β-galactosidase,β -glucuronidase and LAP which are also known to degrade ECM (Adams and Hamilton, 1992). Therefore, macrophages might be a contributor for the LAP in the present study.

Retinoic acid (RA) treatment of the regenerating limb of *Hynobius* is known to cause prolonged dedifferentiation and pattern duplication (Lee and Kim, 1990; Ju and Kim, 1994). Similar results were obtained in the RA-treated limb regenerates of axolotl (Maden, 1982; Scadding and Maden, 1986a). In the immunohistological study of LAP in *Hynobius leechii*, RA was found to enhance and prolong LAP immunoreactivity (Ju and Kim, unpublished data). In the present study, similar profile was also observed.

In the study of LAP expression in RA-treated urodele limb regeneration, one question is whether RA activates the LAP activity or increase its level. In some cases, lysosomal enzyme activities have been known to be elevated by RA and its derivatives. Roles et al. (1969) reported that vitamin A caused destabilization of lysosome, leading to tissue necrosis by stimulating exocytosis of lysosomal enzymes. In the regenerating limbs of Mexican axolotl, the expression of LAP was found to be elevated at dedifferentiation stage. Therefore, the increased expression of LAP might be partially responsible for the remodeling of extracellular matrix (ECM) during dedifferentiation process.

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