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Full Length Research Paper

Exogenous NGF favors initiation of lizard tail regeneration while EGF and TGF-β truncate regenerative growth and commit to precocious muscle and cartilage differentiation

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Since growth factors exert control over various cellular events during development and as information in this regard on regenerative growth are scant, the present study has evaluated the effect of exogenous *in loco* administration of EGF, TGF-β and NGF during the first few days post-autotomy on the course of tail regeneration in *Hemidactylus flaviviridis.* Further evaluated is the efficacy of NGF to rectify the retardatory influence of hypothyroidism on regeneration. This study reveals that, EGF and TGF-β inhibit tail regeneration while, NGF stimulates it. Histologically, the EGF treated lizards showed greater collagen formation and precocious epithelial and myogenic differentiation. TGF-β had inhibitory influence on dedifferentiation while favoring preponderant chondrogenic differentiation. Overall, the present study on the differential effects of growth factors on various aspects of cell proliferation and differentiation, suggest the need for various growth factors on a precisely synchronized temporal and spatial order for a normal regenerative growth.

Key words: *Hemidactylus flaviviridis*, regeneration, nerve growth factor, fibroblast growth factor, transforming growth factor-β.

INTRODUCTION

Restoration of lost or damaged tissues or organs due to natural causes, disease or accident is the central theme of regenerative medicine. Though tissue engineering and stem cell biology are very much part of the approaches for the same (Stocum, 2004), understanding of ontogenic developmental events and tissue turn over in adult animals can provide great inputs to approaches in regenerative medicine (Rodtke and Clevers, 2005). An alternative approach involves applying the principles of inherent regenerative capacity of non-mammalian models, essentially the molecular events that permit tissue regeneration. Many of the sub mammalian vertebrates from fishes to reptiles demonstrate exemplary ability to replace lost body parts (Tsonis, 2000). An incidental interest in regeneration is the possibility of finding some clues for the causative mechanisms of cancer as the early phases of regeneration marked by dedifferentiation and proliferation of cells bear close resemblance to oncogenesis. Apart from being a fascinating biological phenomenon, regeneration has also attracted biomedical interest for its potential to replace old or damaged tissues with new ones. Most of the studies on regenerative biology aimed at biomedical applications have focused on stem cells *in vitro* but understanding of regeneration requires *in vivo* studies, as complex interactions and communications within and among the different cell types characterize the process. Model organisms are essential for such *in vivo* studies, which can provide us with necessary information needed for eventual manipulation and control of regenerative properties. The importance of studying the process in lizards in the above context needs no elaboration when we consider the fact that, reptiles represent the closest

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ancestral stock from which the homeotherms have evolved. Nevertheless, reptilian regeneration is a neglected field and apart from us, only Alibardi (2010) has taken up studies on this aspect in recent times.

Though neglected in comparison to regeneration studies in fishes and amphibians, such studies using lizard tail as a model have been in vogue in this laboratory for nearly four decades. Epimorphic regeneration as exemplified by the lizard tail comprises of post-autotomy preparation phase and a redevelopment phase (regressive and progressive phases) characterized by a non-scarring type of wound healing, blastema formation (akin to a tail bud), re-differentiation and growth phases (Ramachandran, 1996). Other studies have established local histological as well as adaptive biochemical and metabolic alterations in the geckonid lizard, *Hemidactylus flaviviridis* and the scincid lizard, *Mabuya carinata*. Even adaptive systemic responses as well as hemodynamic alterations have also been documented (Shah et al., 1977, 1980, 1982; Ramachandran, 1996; Ramachandran et al., 1979, 1985). Since the establishment of regeneration blastema is crucial for regenerative growth, immediate post autotomy periods deserve attention as a phase of molecular intricacies setting up an appropriate environment for the initiation of regeneration. A number of synchronized inter-related molecular events involving cytokines, growth factors, hormones and modifications of extracellular matrix (ECM) are likely to trigger adaptive changes forming the core of regressive phase changes.

In this context, two of our recent studies have evaluated the participation of signaling molecules and ECM remodeling in the immediate periods subsequent to caudal autotomy (Deshmukh et al., 2008; Nambiar et al., 2008). The above studies showed subtle temporal alterations in signaling molecules, second messengers and enzymes of ECM remodeling. Regeneration of a complex heterogeneous organ like the lizard tail, involving non-scarring wound healing, multiple cellular events, ependymal outgrowth and epithelio-mesenchymal interaction leading to progressive differentiation of heterogeneous tissues in a proximo-distal order, is likely to involve controlling/ regulating molecules like neurotrophic factors, and growth factors in the molecular ecology of the regenerating tail. As of now, understanding of the involvement of such factors in the reptilian regenerating system is poor. Local application is one of the simplest modes of study to understand the role of growth factors.

Hence the present study evaluates the influence of exogenous administration of nerve growth Factor (NGF), epidermal growth factor (EGF) and transforming growth factor-β1 (TGF-β1) on the course of tail regeneration in terms of wound healing, blastema formation and differentiation. Since both thyroxine and NGF are important for the development of nervous system and as thyroxine reportedly increases NGF content (Walker et al., 1979), the study also assesses the influence of NGF given to hypothyroid lizards to decipher their inter-relationship if any.

MATERIALS AND METHODS

Chemicals

NGF was purchased from Sigma Aldrich, USA, while TGF-β was a gift from Department of Endocrinology, PGIBMS, Chennai and EGF, a gift from Division of Biotechnology, Department of Microbiology, MS University of Baroda, India.

Experimental animals

Adult *Hemidactylus flaviviridis* (10±2 g) of both sexes with snout - vent length of 70 to 80 mm were used for the experiments. The cages housing the animals measured 18 × 15 × 10 with one side of transparent glass and ventilated on three sides. Each cage housing six lizards was balanced for size. The animals were maintained under a normal light-dark photoperiodic schedule and were fed on nymphs and water *ad libitum.* Autotomy was performed by pinching off the tail three segments from the vent

Experimental schedules

Set I: Evaluation of influence of NGF, EGF and TGF-β

Sixty lizards were divided into 6 groups of 10 each.

Group 1(Control): The tail of these lizards was autotomised three segments distal to the vent and injected with 0.6% saline at the cut end of the tail for 15 days.

Group 2 (NGF treated): The tail of these lizards was autotomised three segments distal to the vent and injected with 10 ng of NGF *in loco* for three consecutive days post autotomy.

Groups 3 and 4 (EGF treated): The tail of these lizards was autotomised three segments distal to the vent and injected with 5 or 10 ng of EGF respectively for ten consecutive days post autotomy.

Groups 5 and 6 (TGF-β treated): The tail of these lizards was autotomised three segments distal to the vent and injected with 5 ng TGF-β1 for ten consecutive days post autotomy (Group.5). Group 6 animals were treated with 5 ng TGF-β1 after the formation of blastema (10 to 12 days) for five consecutive days.

Set II: Evaluation of the influence of hypothyroidism, thyroxine replacement or replacement with NGF

40 lizards were divided into 4 groups of 10 animals each.

Group1 (Control): This group of lizards served as euthyroidic control. They were force fed with 0.6% saline on every alternate day. At the end of the 5th dose of saline, the tail was autotomised. Saline feeding was continued until the end of the experimental period.

Group 2 {(Hypothyroid (HT)}: These lizards were rendered hypothyroidic by force-feeding 0.1% 6-propyl, 2-thiouracil (PTU). The tail of these lizards was autotomized at the end of 5th dose of PTU and PTU feeding was continued every alternate day until the end of the experiment.

Table 1. Number of days taken to attain various arbitrary stages in control and experimental lizards.

Manipulation	WH	PB	B	IG
Control	7	9	10	11
EGF	6	11	13	17
TGF-β	7	13	17	-
NGF	5	6	7	8

WH; Wound Healing, PB;Preblastema, B; Blastema, IG; Initiation of growth, EGF; Epidermal growth factor, TGF-β; Transforming growth factor, NGF;Nerve growth factor, T4-Thyroxine.

Group 3 (Thyoxine replacement {TR}): These lizards were also rendered hypothyroid and their tail was autotomized at the end of 5th dose of PTU. Subsequent to autotomy, PTU feeding was continued every alternate day. Apart from this, they were also injected with 10µg of thyroxine *in loco* every alternate day starting from 3 days post autotomy and continued until the end of the experiment.

Group 4 (NGF replacement): These lizards, as those of Group 2, were also rendered hypothyroid and their tail was autotomized at the end of the 5th dose of PTU. Five injections of 10 ng NGF were given *in loco* for 5 consecutive days post autotomy. PTU feeding was continued until the end of the experiment.

Parameters evaluated

Subsequent to treatment with NGF, EGF and TGF-β, the number of days taken to reach the various arbitrary stages of regeneration like, wound healing (WH), preblastema (PB), blastema (B) and initiation of growth (IG) was noted. Since there was inhibitory influence of EGF and TGF-β, the tails of these lizards were cut and processed for histological observations. Prior to dehydration and embedding in paraffin, the tails were decalcified and sections of 3 to 5 micron and 8 to 10 micron thickness were cut and stained with Casson's or Masson's trichome. Some sections were also stained with PAS staining. Since NGF treatment has a stimulatory influence on regeneration, the length of tail regenerated in control, NGF, HT, TR, and HT+NGF groups of lizards was measured every alternate day. The growth rate per day and total replacement of tail were also calculated.

Statistical analysis

The values are expressed as Mean±S.E.M for n = 10. The data was subjected to student's t test and to Duncan's multiple range test.

RESULTS

EGF and TGF-β1 treatment

As shown in Table 1, treatment with both EGF and TGF-β1 resulted in inhibition of regeneration. Though EGF promoted earlier wound healing, the attainment of preblastema and blastema stages was delayed (Figure1).

Figure 1. Photomicrographs of control (a) 1 ng EGF treated (b) 5 ng EGF treated, and (c) lizards, showing the dose dependent inhibitory influence of EGF at the end of 15 days.

In contrast, though TGF-β1 did not show any effect on WH, the formation of PB and B stages was significantly delayed and a poor blastema was formed. However, lizards injected with TGF-β1 after the formation of a blastema did not show any effect and they regenerated their tail at the same rate as the controls.

Histological observations

Control lizards in the wound healing stage showed a single layered wound epithelium with no collagen material and the pre-blastemic and blastemic stages were characterized by a thickened blastemic epithelium with no collagen material and compactly packed mesenchymal cells. Differentiation stage was marked by central cartilaginous neural tube with ependyma inside and differentiating muscle bundles on the lateral sides and differentiating integument (Figure 2).

Histologically, the EGF treated lizards showed heavily deposited collagen below the epithelium (Figure 1). In those cases where regeneration proceeded up to pre-blastema stage, showed thickened epithelium with tendency for keratinization and scale formation. In many cases, specific staining indicated formation of collagen below the blastemic epithelium and even in the mesenchymal mass. Precocious and premature differentiation of muscle elements could be clearly seen even extending up to the distal tip close to the apical epithelium (Figure 3). Lizards given only 1 ng EGF or even those lizards which were given 5 ng EGF, where some growth occurred after the stoppage of EGF treatment, tended to show a hooked stumpy growth and, in these cases, PAS staining indicated less GAG

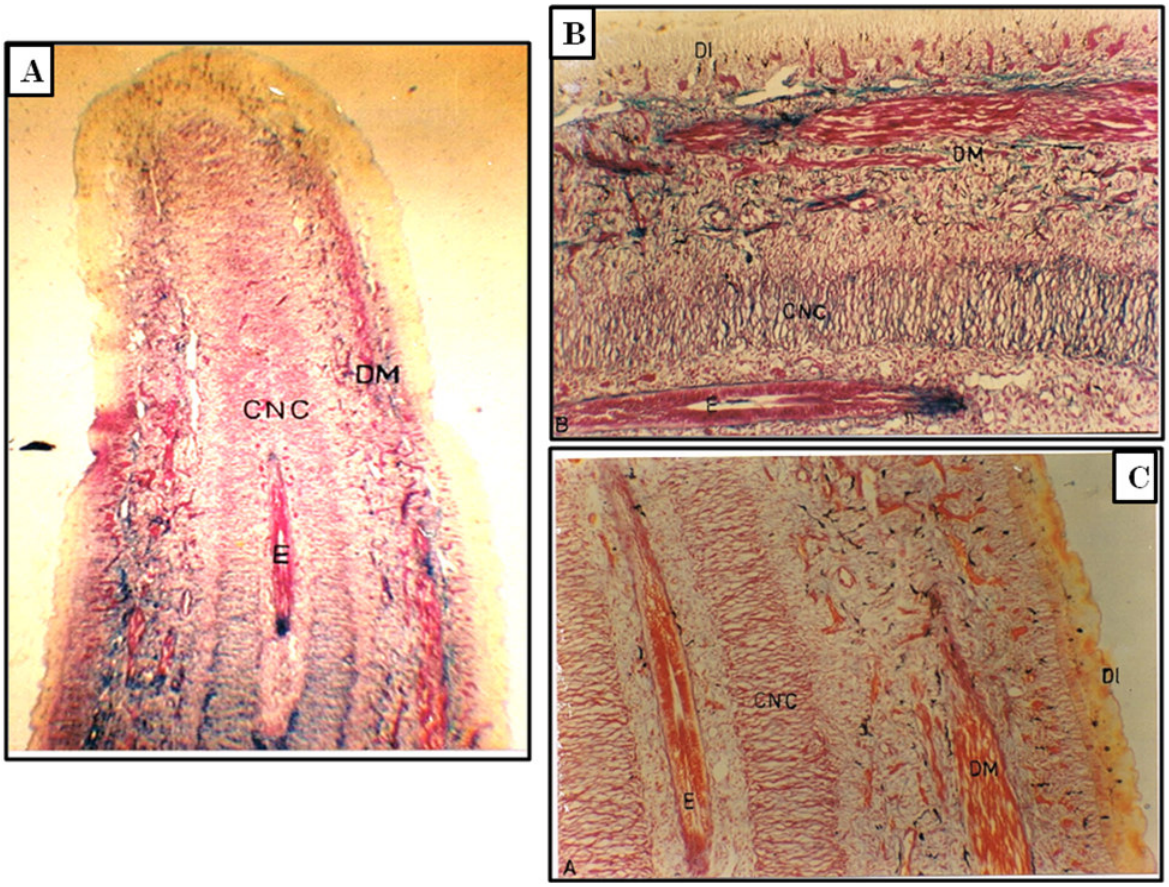

Figure 2. Photomicrographs of a section of regenerating tail of control lizard in the differentiation stage stained with Casson's stain showing various differentiating elements (A), enlarged version of Casson's stained section showing the lateral half with differentiating muscle (DM) and ependyma (E). Integument appears yellow, muscle and ependyma appears orange and cartilaginous neural canal appears pink (B) and central part of section of differentiating tail stained with Mallory's triple stain showing differentiating integument (DI), differentiating muscle (DM), cartilaginous neural canal (CNC) and ependyma (E). Differentiating muscles and ependyma appears red and collagen appears green (C).

substances in the differentiating cartilaginous tube with extensive muscle differentiation all over the tip (Figure 4). Erythropoeitic activity was greatly stimulated in the adipose tissue of the stump tail and large clumps of blood cells could be seen accumulated in large numbers at the cut end below the epithelium (Figure 5). Differential staining revealed increased GAG deposition and precocious and preponderant chondrogenic differentiation (Figure 6). Muscle differentiation was totally inhibited.

NGF treatment

NGF treatment resulted in faster wound healing and early blastema formation and initiation of growth. There was sustained faster rate of growth that resulted in 20% replacement of lost tail within 25 days, as compared to 14% in the control lizards (Table 1).

PTU treatment

PTU induced hypothyroidic lizards showed delayed WH, BL formation and IG. The total length of tail regenerated was significantly less compared to euthyroidic controls and the total replacement at the end of 25 days was only 5%.

T4 and NGF replacement

All the retardatory influence of hypothyroidism was completely nullified by either T4 or NGF replacement. WH, BL formation and IG occurred at periods very much comparable with those noted for the control lizards. The total length of tail regenerated and the total percentage replacement at the end of 25 days were much closer to those of the controls though slightly lesser. On a comparative basis, NGF replacement seems to be a shade

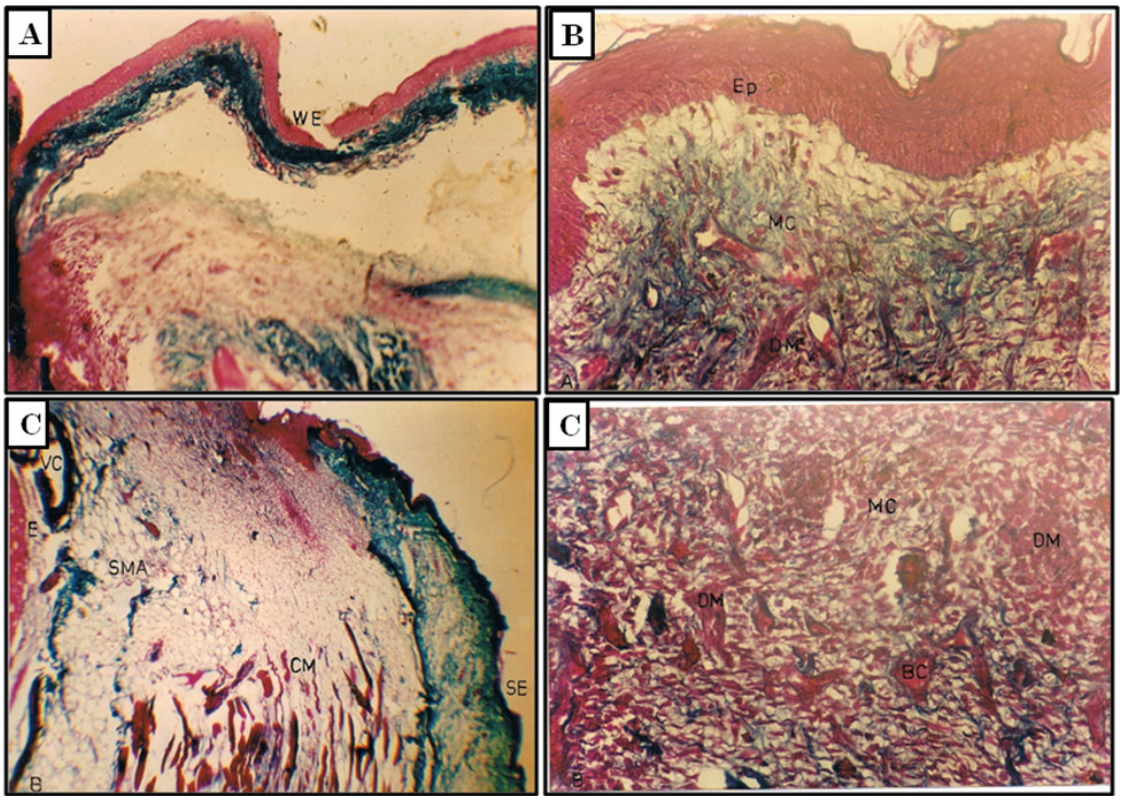

Figure 3. Photomicrographs of the (A) Mallory's triple stained sections of tail of experimental lizards treated with EGF in the wound healing stage showing deposition of thick layer of collagen (green) below the thickened epithelium (WE), (B) enlarged view of the pre-blastema showing presence of collagen (greenish blue) below the thickened epithelium (EP), mesenchymal cells (MC) and precocious differentiating muscle cells (DM), (C) lateral half of the above tail section showing the presence of thick collagen (green) below the stump epidermis (SE), sub muscular adipose tissue (SMA), vertebral column (VC) and part of ependyma (EP) and, (D) enlarged version of the mesenchymal mass showing loose mesenchymal cells (MC), blood cells (BC)-red, differentiating muscle mass (DM)- red and collagen material (green).

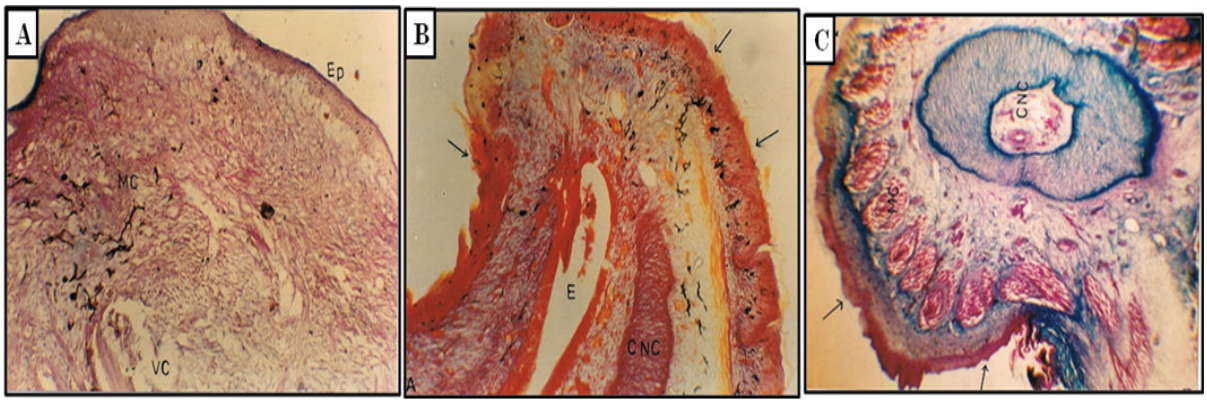

Figure 4. photomicrographs of (A) PAS stained section of a regenerating tail arrested at the pre-blastema stage from a lizard treated with EGF showing loose mesenchymal cells (MC), thick epithelium (Ep) and some GAG material (purplish in color) only around the vertebral column of the tail stump but not in the differentiating cartilage area, (B) hooked tip of the regenerating tail of EGF treated lizard showing precocious differentiation and keratinization of the epidermis (arrow), cartilaginous neural canal (CNC) and ependyma (E) in Mallory's triple stained sections and (C) the tip of a hooked tail of EGF treated lizard showing precocious epidermal differentiation arrows and muscles (MC), cartilaginous neural canal (CNC) and a heavy deposition of collagen (greenish-blue) below the epidermis and around the muscle bundles as well as in the cartilaginous neural canal in Masson's trichome stained sections.

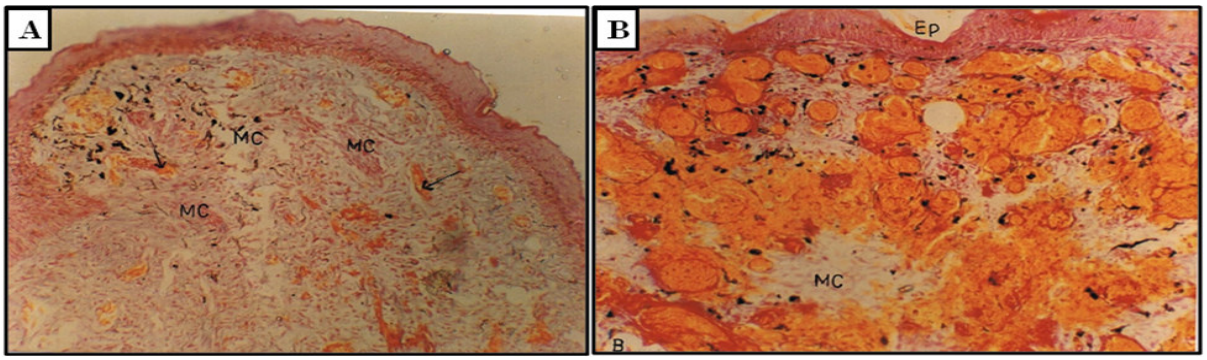

Figure 5. Photomicrographs of TGF-β treated lizards tip of the blastema (A) showing loose mesenchymal cells (MC), scattered accumulation of blood cells (arrow, yellow color), some undifferentiated muscle fibres released from the cut end of the stump (MC) and cartilage condensation in the centre, (B) the tip of the pre-blastema showing epithelial (Ep) and loose mesenchymal mass (MC) studded with clumps of erythrocytes (yellow color).

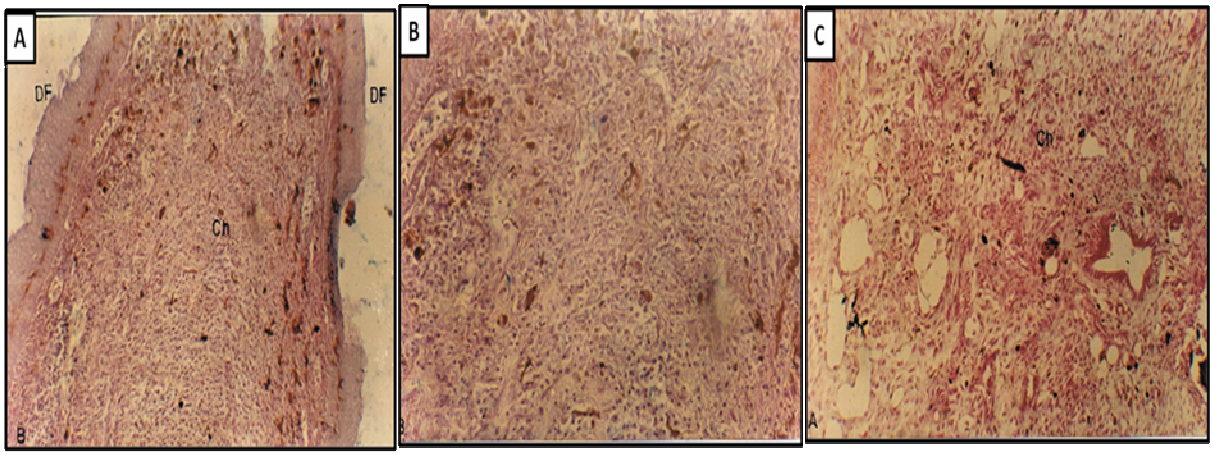

Figure 6. Photomicrographs of sections of regenerating tail of a lizard treated with (A) TGF-β showing only chondrogenic differentiation (Ch) in the mesenchymal tissue and GAG material (purple color) in Casson's stain, (B) section of a regenerating tail of lizard treated with TGF-β1 showing extensive chondrogenesis with no other tissue differentiation. The purplish background represents GAG material.

Table 2. Number of days taken to attain various arbitrary stages in control and experimental lizards.

Manipulation	WH	PB	B	IG
Control	7	9	10	11
NGF	5	6	7	8
PTU	10	11	13	14
PTU+T4	9	10	11	12
PTU+NGF	8	9	10	11

WH; Wound Healing, PB; Preblastema, B; Blastema, IG; Initiation of growth, NGF; Nerve growth factor, PTU;6-Propyl-thiouracil, T4-Thyroxine.

more effective than T4 replacement (Table 2).

DISCUSSION AND CONCLUSION

Of the three growth factors tested, EGF and TGF-β1, show inhibitory effects on regeneration while, NGF depicts significant favorable response. The only study on growth factor in lizard tail regeneration is of Alibardi and Lovicu (2010) who have immunolocalized FGF1 and FGF2 in the regenerating tail of *Lampropholis guinchenoli*. This study conducted in post blastemic regenerating tail suggests some role for FGFs in tissue differentiation especially of muscle and skin. They suggested a possible neurotrophic role for FGF2 in the light of its localization at the growing ends of spinal cord and nerves. Other studies on regenerating systems of fishes and amphibians suggest roles for FGFs in proliferation of neural progenitors and the ependymal

cells of the spinal cord (Zhang et al., 2000) and in epithelio-mesenchymal interactions (Bouzaffour et al., 2009). The interactions of FGF2-FG1 seem akin to FGF8-FGF10 interactions in the developing limb bud (Martin, 2001) and, FGFs seem to be good evocators of wound healing and regeneration.

EGF is a small single chain polypeptide that stimulates the proliferation of epidermal and epithelial cells in whole animals and a variety of cell types in culture and controls many other functions (Carpenter, 1993; Wong and Gillaud, 2004). In keeping with its role in epithelial cell proliferation, EGF treatment in the present study promoted early wound healing and helped form a thickened multilayered blastemic epithelium, with delayed blastema formation and stunted growth of the regenerate thereafter. This post-blastemic inhibition on regenerative growth appears related with the heavy deposition of collagen below the epithelium. It is well established that during regenerative wound healing, the deposition of sub-epidermal collagen and/or scar tissue formation do not occur unlike in non-regenerative wound healing (Shah et al., 1980; Harty et al., 2003; Nambiar et al., 2008). Though the deposition of connective tissue material occurs on the lateral sides of regenerate below the differentiating epidermis, it never occurs under the apical wound or blastemic epithelium thereby permitting the continued elongation of the regenerate. The deposition of dermal connective tissue on the lateral sides of regenerate in fact promotes differentiation of integument marked by scale formation and keratinization (Alibardi, 2010). Apparently, precocious and premature epithelial differentiation into scales and keratinization visible even at the apical region in EGF treated lizards finds correlation with the deposition of dermal substance all around the blastemic epithelium. The present observation of EGF induced collagen formation and deposition under the wound epithelium, finds support in the many reports of EGF's role in collagen synthesis under both *in vivo* and *in vitro* conditions (Laato et al., 1987; Babul et al., 2004; Berlanga-Acosta et al., 2009). Another prominent influence of EGF seen in the present study is the precocious and preponderant myogenic differentiation resulting in increased muscle bundles below the incipiently formed blastemic apical epithelium. This myogenic effect of EGF finds support from the observations of Lim and Hauschka (1984), and Olwin and Hauschka (1984) of proliferation of myoblasts in presence of EGF and the loss of receptors for EGF with the fusion of myoblasts and their differentiation into myotubes. Further, Yamane et al. (1997) have shown involvement of EGF in myogenesis of tongue epithelium and induction of myogenic genes (myf5) and myogenic transcription factors. Apparently, as in mammals, EGF is able to promote muscle differentiation in reptiles as well. Another observation of some merit is the decreased amount of glycosaminoglycan (GAG) material in the matrix of differentiating cartilaginous tube and the hooked nature of the regenerate with stunted growth.

TGF-β is a member of the family of polypeptides, which seems to regulate cellular activity in various metazoan organisms (Herpin et al., 2004). A very important action of this factor is on extracellular matrix synthesis and maintenance and, depending on the cell type, it may exert stimulatory, inhibitory, biphasic or no effect on cell proliferation (Moses et al., 1987). Though TGF-β is capable of stimulating the growth of fibroblasts and osteoclasts, it inhibits the proliferation of many epithelial cells and inhibits growth of developing mammary gland (Pfeilschifter et al., 1990). Another function accredited to this factor is in the various facets of wound healing, which led to its consideration as a potential therapeutic agent for wound healing (Mustoe et al., 1987). Since TGF-β3 is potent in epithelial proliferation and wound healing, TGF-β1 used in the present study did not hasten post-caudal autotomy wound healing. In fact, TGF-β3 is involved in scar less wound healing (Kohama et al., 2002). The most conspicuous effect of TGF-β1 treatment in the present study is the formation of a poor regeneration blastema after a pronounced delay. Further, differential staining techniques revealed increased GAG deposition in mesenchymal mass. This observation is in agreement with the reported effect of TGF-β in stimulating the synthesis of all major matrix proteins, such as collagen and fibronectin (Ignotz and Massague, 1986), tenascin (Pearson et al., 1988), elastin (Liu and Davidson, 1988), glycosaminoglycan (Chen et al., 1987) and thrombospondin (Penttinen et al., 1988). The formation of a poor blastema as well as the inhibition of post-blastemic growth could be due to the inhibitory influence of this peptide on differentiation/ recruitment of cells, as has been observed presently, and on proliferation of the dedifferentiated cells. The purported inhibition of proliferation of dedifferentiated cells is well neigh possible as TGF-β is reportedly a potent inhibitor of growth of a wide variety of cells (Holley et al., 1980; Proper et al., 1982; Lawrence et al., 1984; Roberts et al., 1985). A related observation is the inhibition of DNA synthesis in hepatocytes under the influence of TGF-β in normal and regenerating liver of rat (Strain, 1990). Other observed anomalies consequent to TGF-β1 treatment are inhibited myogenesis and precocious chondrogenesis. These effects appear to be akin to that reported for mammals, as TGF-β purportedly stimulates chondrogenic differentiation and inhibits adipogenic and myogenic differentiation (Pfeilschifter et al., 1990; Miyazono, 2000; Sekiya et al., 2002; Ramón Ríos et al., 2002; James et al., 2009; Kim et al., 2009). Importance of TGF-β in multiple events during regeneration stands validated by the studies of Ho and Whitman (2008), and Tseng and Levin (2008) wherein they have shown the localization of key components of the TGF-β signaling pathway and blockage of Xenopus tail regeneration at multiple points, on inhibition of TGF-β signaling by the use of a specific inhibitor. Apparently, a generalized inhibition of all TGF-β

fractions affects multiple events and, the blockage of regeneration suggests the important role of this growth factor in regenerative mechanics. A novel feature observed in the present study with reference to TGF-β1 action is the stimulated hematopoietic activity, predominantly eryhtropoetic, in the adipose tissue of the tail stump, as marked by congregation of RBCs in the loose mesenchymal mass of the poorly formed blastema. In the wake of the hitherto unreported functional involvement of TGF-β in erythropoeitic activity, the present observations implicate this growth factor in such a functional involvement in lower vertebrates

As against the inhibitory actions of EGF and TGF-β1, NGF showed stimulatory influence on regeneration. Administration of NGF in the tail stump, post caudal autotomy, significantly reduced the latent period (by 3 days) in blastema formation and provided greater momentum to growth in the initial phase. This is borne out by the significantly pronounced growth rate recorded during the first 10 days post autotomy. Presumably, the lizard tail has the potential to respond to exogenous NGF or related peptide, and initiate regeneration. The observation of early wound closure in NGF treated lizards indicates its role in promoting wound healing that stands well supported by other studies (Werne and Grose, 2003). Regenerative growth post autotomy/amputation of lizard and amphibian tail is essentially dependent on ependymal outgrowth from the cut end of spinal cord as a *priori* and its triggering action by way of secretion of MMPs, neurotrophic factors etc, which lead to non-scarring wound healing and formation of a regenerative blastema (Alibardi and Lovicu, 2010). Studies on notochord and tail regeneration have suggested the elaboration of important neurotrophic factors like NGF, BDNF, neurotropins and neurotrophic factors as necessary mediatory messages for triggering the initiation of regeneration (Chernoff, 1996; Ferretti et al., 2003).

A probable clue to the involvement of NGF in the autotomised tail comes from the observation of the ability of exogenous NGF to nullify the delay in blastema formation exhibited by hypothyroidic lizards. Thyroxine stands implicated in the outgrowth of the ependyma "*a priori*" for regeneration to occur as, it is responsible for providing the inductive influence for the initiation of regeneration in lizards (Bellaris and Bryant, 1985). Our previous studies (Ramachandran et al., 1984; Ramachandran and Abraham, 1990; Ramachandran and Kurup, 2006) have indicated hypothyroidism induced delay in outgrowth of ependyma to be responsible for retarded regenerative growth. However, in the present study, exogenous NGF could compensate for the lack of thyroxine and initiate a normal regenerative outgrowth (even better than that shown by T4 replacement). This provides substantial circumstantial evidence for ependyma as a source of NGF in the lizard tail. Two relevant observations that lend credence to this assumption are the ultra-structural changes suggestive of secretory activity of ependymal tip (Alibardi, 2010) and the ability of thyroxine to increase NGF content (Walker et al., 1979). Further evidence comes from the reported expression of NGF receptors in the central nervous system subsequent to the injury of spinal cord (Brunello et al., 1990). The NGF elaborated by the ependyma may also be implicated in neurite outgrowth and other normal functions associated with NGF such as accumulation of neurofilaments, formation of microtubules and axonogenesis. It is also likely that, NGF may induce an environment conducive for regeneration by ECM remodeling by way of expression of MMPs and favor angiogenesis and mitotic activity in the apical epithelial cap. It can be presumed that, the ependyma, under the influence of thyroxine produces NGF required for neurite outgrowth and the formation and action of the mitogenic factor (s) which would contribute to continued regenerative growth.

Overall, the present study has revealed a positive influence of NGF on tail regeneration and a negative effect of both EGF and TGF-β1 by their multiple cellular and sub cellular effects. It is inferable that, uncontrolled or unscheduled expression of EGF and TGF-β temporally or spatially can lead to aberrant regeneration with singular tissue differentiation, cautioning the application of growth factors in regenerative medicine for regrowing or repairing organs or structures comprised of heterogeneous tissues.

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