Axolotl Xenografts Improve Regeneration of Xenopus Hind Limbs

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Axolotls regenerate perfect copies of amputated limbs, whereas Xenopus froglet limbs regenerate only a spike of cartilage. We asked whether axolotl muscle and cartilage xenografted from normal or GFP-labeled limbs to amputated froglet limbs, with or without treatment with cyclosporin A (CSA) and/or retinoic acid (RA), would improve Xenopus limb regeneration via the release of regeneration-promoting factors into the host limb tissue. The grafted froglet limbs were allowed to regenerate for three months to two years. We detected initial symptoms of graft vs. host disease with or without CSA treatment that subsequently disappeared. The grafted limbs first formed a spike that subsequently grew wider at the tip and after three months began to separate into 2-5 digit-like structures that continued to grow. CSA and low-dose RA treatment decreased the time at which digit formation could be detected but were not necessary for digit formation. The digit pattern was not asymmetric, thus individual digits were not identifiable. Immature muscle was detected in the regenerated limbs by trichrome and MF-20 antibody staining, and nerve fibers were detected by Luxol Fast Blue staining. In one limb with a GFP graft, a few axolotl cells were detected around the base of the digits that may have stimulated digit separation. Although the mechanism of digit formation remains obscure, we conclude that factors released by degraded axolotl tissue or surviving axolotl cells can stimulate complex tissue regeneration and initiate the first step of digital anterior-posterior pattern formation in regenerating Xenopus hind limbs. These results have significance for the possibility of stimulating the regeneration of complex mammalian structures that have been injured by trauma or disease.

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