thalidomide's teratogenic action, also based on a lack of cells in the proximal region of limb buds. An important prerequisite of limb formation in the very early stages is the migration of cells from the somatopleura (for the skeleton and connective tissue) and from the somites (for the musculature) to the sites of the prospective limb buds[7]. Inhibition of these processes by thalidomide would lead to a reduction of cells that may later react to the signals of the AER. The somatopleuric mesenchyme of the prelimb or early limb region of rhesus monkeys (*Macaca mulatta*) has already been proposed to be the site of thalidomide's action[8].

We have suggested that thalidomide inhibits cell migration before the AER starts to function. This idea was based on the finding that thalidomide downregulates adhesion receptors, including some integrins, on the cell surface of the early primate embryo *in vivo*[9,10]. Such adhesion receptors are not only involved in cell–cell interactions, which are known to be essential for morphogenetic differentiations in the embryo, but also take part in interactions between cells and the extracellular matrix[11]. This could form the basis for an inhibition of cell migration. The downregulation of adhesion receptors occurs only in the primate's primordia that are susceptible to the action of thalidomide, such as the limbs and heart, but not, for example, in the brain. Furthermore, this effect on adhesion receptors occurs in primates but could not be demonstrated in rodent embryos. Our data therefore fulfil all the criteria we have listed above.

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Tabin replies — Neubert *et al.* suggest some useful criteria for evaluating hypotheses for the teratogenic activity of thalidomide. However, these criteria are misapplied in their critique of my explanation of thalidomide-induced limb defects, in part because of misunderstandings regarding the current literature on limb development.

I suggested that phocomelia (missing proximal limb elements) results from lack of progress-zone proliferation in the context of continued distalization by fibroblast growth factor (FGF) signalling[1]. Neubert *et al.* consider this suggestion implausible because the established period of thalidomide susceptibility in marmosets (starting at stage 11, equivalent to stage 16/17 in the chick) is before the formation of the apical ectodermal ridge (AER), the structure at the distal tip of the limb bud that is the source of FGFs. But what matters is not when a morphological AER forms, but when FGFs are first produced at the distal tip.

In all species examined, including mice, chicks and frogs, FGF-8 is first expressed in a stripe of ectoderm corresponding to the future AER just before the initial outgrowth of the nascent limb bud[2,3], at stage 16 in the chicken or what would be early stage 11 in the marmoset. The AER forms later, and at different relative times in different species, arising later in the mouse than in the chick, and not at all in amphibians. The stages of limb development at which marmosets are susceptible to thalidomide, as previously defined by Neubert and co-workers[4], correspond extremely well to the stages of limb development when proximal limb elements are specified under the influence of the distal ectoderm[5], lending explicit support to my proposal.

Neubert *et al.* also criticize my model for failing to explain other aspects of their criteria. But I never intended to address the pharmacological basis of thalidomide's effects, and indeed, as I pointed out, the pharmacology of this compound remains controversial. Whatever the mechanism, it undoubtedly has similar cellular effects in different regions of the embryo. However, in the context of the limb bud, the consequence of this is an ultimate decrease in growth of the proximal limb elements, and the point of my hypothesis was to explain in developmental terms specifically why proximal elements should be missing. This is a distinct question from the other important aspects of Neubert *et al.*'s list of criteria, such as the specificity of species susceptible to the drug or the specific set of organs affected, which probably involves factors such as differential metabolism of thalidomide.

Neubert *et al.* propose an alternative hypothesis to mine. They suggest that thalidomide could cause its characteristic limb defects by blocking cell migration. However, consideration of limb development suggests that this is unlikely because limb-bud initiation does not involve significant migration of lateral plate mesoderm and, even if it did, blocking such migration would not lead to phocomelia.

They cite results[6] suggesting that limb-bud formation depends on cell migration from the somatopleura and from the somites. But the work cited actually shows that there are two distinct sets of limb precursors (myogenic cells from the somites, and connective tissue or chondrogenic cells from the somatopleura) and that the myogenic cells migrate. It does not provide any evidence that somatopleural cells migrate. Indeed, subsequent work has shown that they do not[7]. Rather, the limb bud forms by local proliferation of the somatopleural mesoderm. Only the myoblasts migrate.

However, even if thalidomide were to block myoblast migration completely, it would not affect the proximodistal pattern of the limb, as removal of all myogenic precursors results in a limb with a normal skeletal pattern but no muscle[8]. Moreover, even if, despite data to the contrary, a migratory block were to decrease the number of somatopleurally derived cells as well as myogenic cells in the early limb bud, this would still not result in phocomelia. As has been shown surgically[9] and by various methods including drug treatment, decreasing the number of cells in the early limb bud results in a narrower limb bud and subsequent loss of elements along the anteroposterior axis, but does not affect proximodistal patterning. This contrasts with the result of experimentally preventing proliferation within the progress zone during limb development, which does result in phocomelia[10].

Neubert *et al.* cite the downregulation of integrin expression[11] to support their model. However, integrins are also important mediators of growth control and are key regulators of angiogenesis[12], so these data are compatible with other explanations for the teratogenicity of thalidomide.

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