We examined the reliability of this form of vaccination in "susceptible" BALB/c mice. All 21 mice exposed to 3.3×10^2 promastigotes and all 12 mice exposed to 10^3 promastigotes were rendered resistant, as shown by their response to a subsequent challenge with a normally pathogenic dose. Only 12 out of 16 mice were made resistant after exposure to 10^2 promastigotes. Thus, all 33 BALB/c mice previously exposed to between 3.3×10^2 and 10^3 promastigotes were protected against a normally pathogenic challenge of the parasite.

"Susceptible" individuals or mice have the capacity to mount a protective response but do not do so for regulatory reasons. Patients with cutaneous and visceral leishmaniasis recover after drug treatment and gain resistance to reinfection as the antibody titer decreases and cell-mediated immunity is expressed, as assessed by skin reactivity to parasite antigens (6). BALB/c mice given either irradiation (*17*), cyclosporin A (*18*), or a monoclonal antibody that results in the depletion of $CD4^+$ T cells (*19*) near the time of infection mount a stable and protective cell-mediated response. The implication—that susceptible humans and animals have the intrinsic ability to mount protective responses—underlies attempts to develop universally effective vaccines.

The approach used here should be applicable in the development of vaccines to other pathogens that cause chronic disease and are preferentially susceptible to a specific cell-mediated attack. The dependence of the type of immunity induced on the dose of antigen administered is likely to reflect the existence of different thresholds for different inductive events (*20*). *Mycobacterium tuberculosis* (*21*), *Mycobacterium leprae* (*21*), *Schistosoma mansoni* (*22*), and human immunodeficiency virus type–1 (*23*) are probably preferentially susceptible to cell-mediated attack. We suggest that the strategy of vaccination described here for *L. major* will also apply to these human pathogens.

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Planar Induction of Anteroposterior Pattern in the Developing Central Nervous System of *Xenopus laevis*

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It has long been thought that anteroposterior (A-P) pattern in the vertebrate central nervous system is induced in the embryo's dorsal ectoderm exclusively by signals passing vertically from underlying, patterned dorsal mesoderm. Explants from early gastrulae of the frog *Xenopus laevis* were prepared in which vertical contact between dorsal ectoderm and mesoderm was prevented but planar contact was maintained. In these, four position-specific neural markers (*engrailed-2, Krox-20, XlHbox 1*, and *XlHbox 6*) were expressed in the ectoderm in the same A-P order as in the embryo. Thus, planar signals alone, following a path available in the normal embryo, can induce A-P neural pattern.

Spemann proposed that dorsal mesoderm can induce neural development in the ectoderm of the amphibian embryo in two ways: by vertical induction, in which signals pass from the involuted dorsal mesoderm to overlying ectoderm, or by planar induction, in which signals pass from the dorsal mesoderm to the ectoderm around the dorsal lip (*1*). However, planar induction appeared to be ruled out when Holtfreter found that urodele exogastrulae lack any obvious neural differentiation (*2*). In these abnormal embryos, mesoderm evaginates during gastrulation, instead of invaginating, and fails to make vertical contact with ectoderm, while presumably retaining planar contact. In other studies vertical signals from dorsal mesoderm were found to induce neural development and A-P neural pattern (*3*). Thus, it has been assumed that neural differentiation and patterning are normally induced by vertical signals alone.

Recent results with *Xenopus laevis*, however, provide evidence for planar neural induction. Exogastrulae express two panneural genes, the neural cell adhesion molecule (N-CAM) and the neurofilament-like protein (NF3) (*4*, *5*), as well as the anterior-specific *xhox-3* (6). However, the possibility of vertical induction cannot be fully excluded because it is difficult to observe the internal movements of mesoderm during exogastrulation. To avoid this problem, studies of planar induction have been carried out in planar explants of mesoderm and ectoderm ["Keller" explants (*7*)], in which the absence of vertical contacts has been well documented (*8*). These explants ex-

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press N-CAM and NF3 RNA (*5*), contain neurons (*7, 9*), and undergo substantial neural morphogenesis (*7, 8*). In addition, an epidermal marker, Epi-1, is repressed in planar recombinates of ventral ectoderm and dorsal mesoderm (*10*). Thus, the planar path of induction seems sufficient for general neural differentiation in X. *laevis*.

We have extended these studies with Keller explants to determine whether planar induction is sufficient for the establishment of extensive A-P neural pattern (*11*). In these explants, dorsal mesoderm (with a layer of presumptive archenteron roof endoderm) and ectoderm are isolated as a continuous sheet from the early gastrula before involution of the mesoderm and hence before any possibility of vertical interactions (Fig. 1). They are cultured flat under a glass cover slip until after neurulation, either as a single sheet ("open-face") or as a "sandwich" of two sheets with their inner surfaces apposed. Mesoderm involution is prevented by the pressure of the cover slip. In the absence of involution, the A-P axes of the ectoderm and mesoderm point in opposite directions, with their posterior ends at the common boundary (Fig. 1B) (*12*). In sandwiches, both mesoderm and ectoderm converge and extend, as they do in normal gastrulation (*7, 8*), causing elongation of the future A-P axis. Convergent extension is greatest in the posterior end of the ectoderm, generating a narrow region fated to become spinal neurectoderm (*8*). More anteriorly, the ectoderm is wider owing to progressively less convergent extension. In open-face explants, only the mesoderm undergoes convergent extension (*13*).

Using antibodies to N-CAM protein (*9, 14*) to stain whole sandwiches, we found that N-CAM expression extended from the narrow, presumptive spinal neurectoderm to the wider, more anterior neurectoderm (Fig. 2, A to C), which, by the fate map, corresponds to midbrain and forebrain regions (*12*). This staining demarcates the neural domain in explants because N-CAM is expressed exclusively in developing neural tissue at these stages (*14*). Somitic mesoderm, identified in Keller explants with the use of an antibody to a muscle

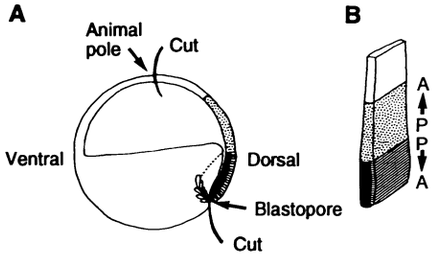

Fig. 1. Diagrams of (**A**) a sagittal section of an early *Xenopus* gastrula (stage 10+), showing region used in Keller explants; from the animal pole to the blastopore, about 60 to 90° wide; (**B**) a single explanted region, depicting A-P polarity as expected from the fate map (*12*). Normal fates in explanted region: white, epidermis; stippled, neurectoderm; black, chordamesoderm and somitic mesoderm; striped, archenteron roof endoderm (*8, 12*).

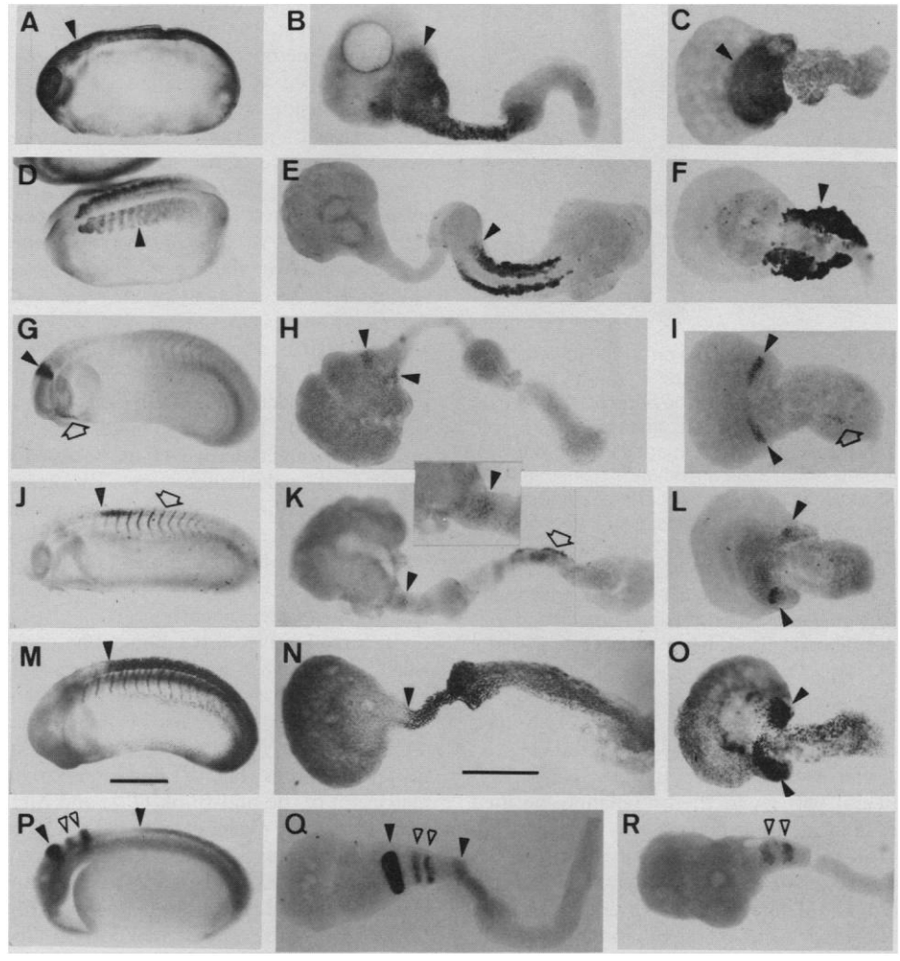

Fig. 2. Expression of neural markers in whole embryos and Keller explants. Left panel: whole embryos, stage 22 to 26, lateral view, anterior left, dorsal up. Center panel: sandwiches, dorsal view, animal pole left. Right panel [except (R)]: open-face explants, dorsal view, animal pole left. Scale bars: 500 μm; scale bar in (M) applies to examples in the left panel and that in (N) to center and right panels. (**A**, **B**, and **C**) N-CAM detected with rabbit polyclonal antibodies to the *Xenopus* 180-kD isoform (*14*) (solid arrowheads), assayed at stage 22. (**D**, **E**, and **F**) Muscle detected with 12-101, a monoclonal antibody to a skeletal muscle–specific protein (*15*) (Developmental Studies Hybridoma Bank) (solid arrowheads), assayed at stage 20. (**G**, **H**, and **I**) *en-2* protein detected with mouse monoclonal antibody 4D9 (*17*), assayed at stage 23 to 24. Solid arrowheads: expression in the CNS. Open arrows: *en-2* expression in (G), the mandibular arch, and (I), the distal mesoderm of an open-face explant. Expression of *en-2* in sandwiches is consistently lower than that in open-face explants. (**J**, **K**, and **L**) *XlHbox 1* protein detected with affinity-purified rabbit polyclonal antibodies to the long form (*18*). Solid arrowheads: expression in the CNS. Open arrows: expression in (J), trunk somites (striped pattern), and (K), somitic mesoderm in a sandwich explant. Somitic mesoderm tends to disintegrate by the stage assayed (stage 26) and is not always present. (K) inset: area of neurectoderm that expresses *XlHbox 1* in sandwiches, approximately twice the magnification of (K). (**M**, **N**, and **O**) *XlHbox 6* protein, detected with affinity-purified rabbit polyclonal antibodies [solid arrowheads point to anterior boundary in (M) and (N)] (*22*), assayed at stage 24. The antiserum cross-reacts with unidentified nuclear antigens in proximal notochord (unpublished observations) and muscle (*19*) and with an extracellular antigen in the cement gland of intact embryos. (**P** and **Q**) *en-2*, *Krox-20*, and *XlHbox 6* RNA detected by whole-mount in situ hybridization, at stage 21. Staining is cytoplasmic. *en-2* and *XlHbox 6* are indicated by solid arrowheads, and *Krox-20* expression by open arrowheads. The *en-2* signal is much greater when this method is used than when antibodies are used; however, the distribution is equivalent. As found with antibodies, *en-2* stains distal (anterior) mesoderm of many sandwiches, although this is not evident (Q). (**R**) Expression of *Krox-20* RNA in the ectodermal portion of a sandwich (indicated by open arrowheads).

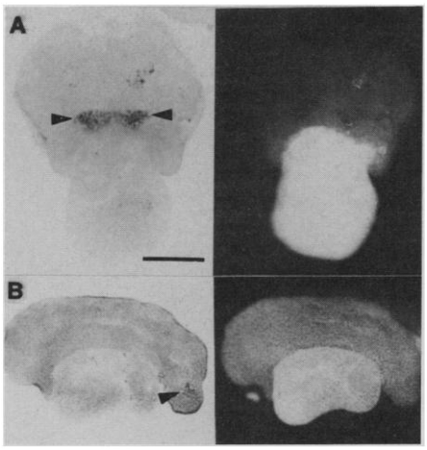

Fig. 3. Expression of (**A**) *en-2* (control stage 23) and (**B**) *XlHbox 1* (control stage 26) in ventral ectoderm grafted onto the edge of FDA-labeled dorsal mesoderm. Left, images viewed with transillumination; right, images viewed with epifluorescence. Solid arrowheads point to *en-2* or *XlHbox 1*–positive nuclei in the ectoderm. In (A), the small patch of staining and fluorescence above the positive nuclei is nonnuclear and is an artifact. The patch of *en-2*–positive nuclei in the lower part of the mesoderm resembles *en-2* expression in the mandibular arch as described in Fig. 1, F to H. Scale bar: 200 μm.

antigen (*13*, *15*) (Fig. 2, D to F), formed two blocks flanking the notochord.

To investigate whether there was A-P neural pattern in these explants, we used immunostaining and in situ hybridization (*16*) to detect the products of three homeobox genes, *engrailed-2* (*en-2*) (*17*), *XlHbox 1* (*18*), and *XlHbox 6* (*19*), and a gene encoding a zinc-finger protein, *Krox-20* (*20*). In intact embryos, these localized to specific regions along the A-P axis of the developing central nervous system (CNS): *en-2* at the boundary of the midbrain and hindbrain (Fig. 2G), *Krox-20* in rhombomeres 3 and 5 of the hindbrain (Fig. 2P), *XlHbox 1* in a broad band in the anterior spinal region (Fig. 2J), and *XlHbox 6* throughout the spinal region (Fig. 2M) and tail bud. These genes were also expressed outside the CNS, as described later.

These genes were indeed expressed in Keller sandwiches in the normal A-P order: *en-2* protein in nuclei anterior to the spinal region (Fig. 2H); *Krox-20* RNA in two bands just posterior to this position (Fig. 2R); *XlHbox 1* protein in the anterior spinal region (Fig. 2K); and *XlHbox 6* protein throughout the spinal region (Fig. 2N), extending into the presumptive posterior mesoderm (*8*). Triply stained Keller sandwiches showed four discrete regions of expression (Fig. 2, P and Q) in the same A-P order as that in the intact embryo. These patterns were observed in the majority of explants [95% for *en-2* (n = 39); 64% for *XlHbox 1* (n = 14); 95% for *Krox-20* (n = 22); 91% for *XlHbox 6* (n = 23)].

Expression in sandwiches could be the result of vertical induction if mesoderm migrates between the two sheets of ectoderm. However, the most migratory mesoderm, head mesoderm, does not induce *en-2* when wrapped with competent ectoderm (*21*). Expression could also be the result of the vertical interaction between the mesoderm of one face of the sandwich and the ectoderm of the other if the two faces are misaligned. To test this, we examined open-face explants. All three homeobox genes were expressed in these explants: *en-2* and *XlHbox 1* in distinct bilateral stripes a short distance from the mesoderm (Fig. 2, I and L), and *XlHbox 6* in larger bilateral regions near to and probably extending into the posterior mesoderm (Fig. 2O) (*Krox-20* was not examined). Thus, these genes were expressed in neurectoderm even in the absence of potential vertical interactions. Patterning occurred in open-face explants without morphogenesis. The pattern was compressed in a way comparable to the fate map (*12*). Thus, the pattern may be set up before convergent extension or independently of it.

In intact embryos, the homeobox genes were also expressed in specific regions outside the CNS: *en-2* protein bilaterally in loose clusters of nuclei in the mandibular arch (Fig. 2G) (*22*); *XlHbox 1* protein in nuclei of somites (Fig. 2J) and lateral plate mesoderm of the mid-trunk (*18*); and *XlHbox 6* protein in nuclei of trunk lateral plate (*19*). Analogous staining patterns were seen in Keller explants: *en-2*–positive nuclei in bilateral patches in the anterior (distal) part of the mesodermal portion (*23*) (Fig. 2, G and I), and *XlHbox 1* expression frequently in nuclei of somites in the middle of the mesoderm (Fig. 2K). These results indicate that the expression of *en-2* and *XlHbox 1* in the neurectoderm and mesoderm occurs independently of the vertical contact that normally exists between these tissues in the intact embryo, contrary to recent proposals (*24*).

Does expression of the homeobox genes in ectoderm occur in the absence of mesoderm? Dorsal ectoderm from early gastrulae (*25*) was isolated without mesoderm and cultured flat as a single layer. Such explants did not express *en-2* (n = 34) or *XlHbox 1* (n = 8). Therefore, contact with dorsal mesoderm is necessary for expression of these genes in dorsal ectoderm.

We further tested whether planar contact is sufficient to induce these genes in ventral ectoderm, which does not normally express them. Dorsal mesoderm from early gastrulae labeled with the lineage marker fluoresceinated dextran amine (FDA) (*26*) was grafted onto the edge of a sheet of unlabeled ventral ectoderm, creating a planar, open-face recombinate (*27*). All three homeobox genes were expressed in the unlabeled portion of these recombinates (6 of 15 expressed *en-2*, 9 of 18 *XlHbox 1*, 2 of 21 *XlHbox 6*) but not in control explants of ventral ectoderm alone (0 of 8 expressed *en-2*, 0 of 21 *XlHbox 1*, 0 of 13 *XlHbox 6*). FDA-labeled mesoderm did not migrate into or mix with the unlabeled ectoderm (Fig. 3, A and B) (*28*). Thus, strictly planar contact is sufficient for induction of these genes, even in ventral ectoderm.

It has been reported that *en-2* protein is not expressed in *Xenopus* exogastrulae (*17*), where planar interactions presumably occur. In extreme exogastrulae there may be partial mesodermalization of the entire ectoderm, owing to absence of the blastocoel, and therefore a loss of neural competence. This possibility has not been examined. Alternatively, inhibitory interactions may occur in exogastrulae but not in Keller explants.

In conclusion, planar signals can induce A-P neural pattern in planar explants of ectoderm and mesoderm, and we propose that this occurs during normal development. Whether the patterning depends on a concentration gradient of inducer (*29*, *30*), homeogenetic induction (*1*, *29*, *31*), ectodermal prepattern (*32*), self-organization (*33*), or some other mechanism, remains to be determined. Vertical signals are also sufficient for neural induction (*3*). Planar signals may be most important early in gastrulation, before extensive mesodermal involution, and vertical signals may subsequently reinforce the early pattern and bring the independently formed A-P patterns of the neurectoderm and mesoderm into register with one another.

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and pharyngeal endoderm (on other side of dashed line) were removed from the inner surface of the chordamesoderm. Eggs were fertilized, dejellied, cultured as in (*34*), and staged according to (*35*). Adult frogs were treated in accordance with University of California guidelines.
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Transforming Growth Factor–β in Leishmanial Infection: A Parasite Escape Mechanism

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The course of infection with the protozoan parasite *Leishmania* is determined in part by their early replication in macrophages, the exclusive host cells for these organisms. Although factors contributing to the survival of *Leishmania* are not well understood, cytokines influence the course of infection. Transforming growth factor–β (TGF-β) is a multipotential cytokine with diverse effects on cells of the immune system, including down-regulation of certain macrophage functions. Leishmanial infection induced the production of active TGF-β, both in vitro and in vivo. TGF-β was important for determining in vivo susceptibility to experimental leishmanial infection.

Parasites of the genus *Leishmania* are responsible for millions of infections in humans, with cutaneous, mucosal, and visceral manifestations. The severity of disease produced by the diverse species that infect humans varies widely, ranging from cutaneous or mucosal involvement that can be self-healing to destructive, to visceral infections that can be mild to fatal. The host or parasite properties responsible for these clinical variations are not known. In experimental models, inbred mice display distinct patterns of susceptibility and resistance to leishmanial infection. For example, *L. major* and *L. amazonensis* can cause self-healing cutaneous infections in C57BL/6 mice but disseminating, fatal infections in BALB/c mice. Another species, *L. braziliensis*, causes cutaneous and mucosal infections in humans but does not cause disease in mice. Host responses that determine whether infections will be healing or nonhealing are not understood, but selective patterns of cytokine production have been associated with murine infections. Thus, infection with *L. major* in susceptible, nonhealing BALB/c mice has a predominantly helper T cell type 2 (T_H2) response, with the production of interleukin-4 (IL-4) and IL-10, whereas infection in resistant C57BL/6 mice results in a predominantly T_H1-type response, with the production of interferon-γ (IFN-γ) and IL-2 (*1–3*). IFN-γ is the cytokine most associated with resistance to leishmanial infection, whereas IL-4 has been linked to a susceptibility to the disease (*1–4*).

Transforming growth factor–β (TGF-β) is a 24-kD protein produced by many cells, including B and T lymphocytes and activated macrophages (*5–7*). It is usually secreted as a latent precursor that requires enzymatic cleavage of carbohydrate groups or transient acidification to release the active cytokine. Among the inhibitory effects of TGF-β on the immune system are decreases in IL-2 receptor induction (*7*) and IFN-γ–induced class II expression (*8*), as well as decreases in IL-1–induced thymocyte proliferation (*9*), B cell differentiation and proliferation (*5*), and cytotoxic and lymphokine-activated killer cell production (*10*). TGF-β also blocks IFN-γ–induced macrophage activation, which diminishes their oxidative responses (*11, 12*). Treatment of macrophages with TGF-β reduces their ability to

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