Can live attenuated virus work as post-exposure treatment?

Sebastian Bonhoeffer and Martin A. Nowak

Simple mathematical models for the competition between different virus variants in the presence of a crossreactive immune response show that, contrary to expectation, selection can favour variants that induce low viral loads. Here, Sebastian Bonhoeffer and Martin Nowak suggest that such 'competitively superior', but 'less pathogenic', mutants may be a possibility for post-exposure treatment of persistent virus infections.

Historically, the most successful vaccines against viral pathogens are those based on live attenuated virus. Such vaccines have played an important role in the eradication of smallpox and in the control of polio in the developed countries[1]. Live attenuated virus vaccines generally elicit a longer lasting, more effective and broader crossreactive protection than do inactivated or subunit virus vaccines. Unfortunately, the live vaccine approach has important disadvantages, as it does not appear to be applicable against persistent and/or oncogenic viruses.

Nearly all current attempts to develop a vaccine against human immunodeficiency virus (HIV) focus on inactivated virus or recombinant virus vaccines[2]. Despite enormous efforts, these attempts have not yet produced satisfactory results. If protection is achieved, it normally occurs only under highly specific conditions: for instance with small challenging doses or with a challenge strain that is highly homologous to the vaccination strain[3–9]. Moreover, many experiments based upon whole inactivated virus have been thrown into question by the recent finding that protection against infection may not have resulted from an immune response against the virus, but rather against components of culture cells that contaminate the virus inoculum[10].

A recent study[11] has kindled a new debate on the value of an approach based on live attenuated virus for the development of an HIV vaccine. This study reported that a live vaccine could successfully mediate protection of rhesus monkeys against challenge with large doses of pathogenic simian immunodeficiency virus (SIV). The live attenuated virus was constructed by deleting the *nef* gene of a pathogenic SIV strain[12]. This mutant was capable of establishing a persistent infection and appeared to be nonpathogenic in all infected animals over the surveyed period of three years. Two years after infection by the attenuated virus, the monkeys were challenged with ten infectious doses of two different pathogenic SIV strain types, the original wild-type virus ($SIV_{mac}239$) or a more distantly related uncloned virus ($SIV_{mac}251$). All four challenged monkeys resisted infection, whereas four unvaccinated control animals died. Two monkeys were later re-challenged with 10^3 infectious doses of the uncloned $SIV_{mac}251$ virus and again resisted infection. In another experiment, in which rhesus monkeys were infected with a different nonpathogenic live attenuated virus, the animals were not protected against subsequent challenge with 10^2–10^3 infectious doses of uncloned virus[13]. Nevertheless, the vaccinated monkeys showed a significant delay in the onset of disease.

The protection conferred by this live attenuated virus seems to be considerably better than that achieved with inactivated virus vaccines to date, but the key problems of vaccination with live attenuated virus remain unsolved. Furthermore, the extended and variable asymptomatic period in HIV will make it extremely difficult to show that an attenuated strain does not cause AIDS (also, even provided that this was shown, the attenuated strain may still induce disease in older people once the immune system starts to decline). Therefore, it seems unlikely that attenuated HIV virus will be tested as a pre-exposure vaccine in the near future.

Post-exposure vaccination?

The use of live attenuated virus as a post-exposure treatment is normally ruled out, since it seems clear that a slow-replicating, attenuated strain can never outcompete an already established, fast-replicating wild type. However, we present here a rationale for post-exposure treatment by live attenuated virus vaccines in an effort to show that competition between different strains in the same patient does not necessarily lead to higher viral burden or increased pathogenicity. A simple mathematical model illustrates that a 'less pathogenic' live attenuated virus may well be superior to the 'more pathogenic' wild-type virus in intra-patient competition, and thereby outperform the wild type, despite the attenuated virus being more-effectively controlled by immune responses.

Our deliberately simple model considers the intra-patient competition between populations of two virus variants: the population of the wild-type virus, v, and the population of the attenuated strain, v_0. Both variants are recognized and eliminated by the same immune response, z. For the sake of simplicity, we will only consider the crossreactive part of the immune responses. (An extension of the model to include

Box 1. An extended model

The following model describes the competition of n antigenically distinct human immunodeficiency virus (HIV) wild-type variants and one attenuated strain in a single host:

$$dv_i/dt = (r - px_i - sz)v_i \qquad \text{where } i = 1,\ldots,n$$

$$dv_0/dt = (r_0 - p_0x_0 - s_0z)v_0$$

$$dx_i/dt = cv_i - (uv + u_0v_0)x_i - bx_i \qquad \text{where } i = 1,\ldots,n$$

$$dx_0/dt = c_0v_0 - (uv + u_0v_0)x_0 - bx_0$$

$$dz/dt = kv + k_0v_0 - (uv + u_0v_0)z - bz$$

Variables: v_i is the population size of each pathogenic variant clone; v_0 is the population size of the attenuated strain; x_i and x_0 are the respective strain-specific immune responses; z is the crossreactive part of the immune response; v is an abbreviation for the total virus population $\sum_{i=1}^{n} v_i$, excluding the vaccinating strain v_0.

Parameters: r and r_0 are the respective replication rates; p, p_0, s and s_0 are the respective immune elimination rates of the strain-specific and crossreactive immune responses; c, c_0, k and k_0 are the respective stimulation rates of the strain-specific and crossreactive immune responses; u and u_0 account for impairment of the immune response due to direct or indirect killing of T helper cells; and b is the natural decay rate of the immune response.

Figure 2 shows the numerical simulation of the above set of differential equations.

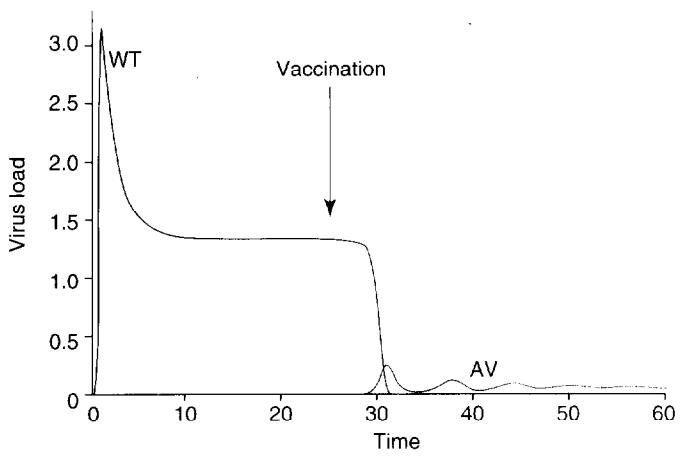

Fig. 1. *A fast-replicating wild-type (WT) virus establishing a high viral load is outcompeted by a slow-replicating attenuated virus (AV) establishing a low viral load. Hence, if pathogenicity is associated with viral load, then the 'less pathogenic' AV can control the disease-inducing WT strain. The viral load of each strain is shown as a function of time (in arbitrary units). At time* t = 0, *the WT strain is introduced, and induces an initial peak of viraemia followed by a persistent infection. The AV strain is introduced at time* t = 25 *and outcompetes the WT strain. Equations 1, 2 and 4 (see text) have been numerically integrated with the following parameters:* r = 20, s = 6, k = 20, u = 6, r_0 = 5, s_0 = 1, k_0 = 2$_0$, u_0 = 1, d = 0.2 *(see text for explanation of parameters). All parameters are given in arbitrary units. Note that the WT strain has a replication rate four times faster than the AV strain, but is more efficiently eliminated by the immune response* ($s > s_0$). *The AV strain is subsequently suppressed to low levels because it is less immunopathogenic* ($u_0 < u$).

strain-specific responses is discussed in Box 1.) In principle, the crossreactive response can be of any form: including a cytotoxic T lymphocyte (CTL) response, an antibody response, an innate immune response or a combination of these responses. The rate of change of the population size of a virus variant is simply births minus deaths. As a first approximation, the birth term is the product of the actual virus population size (v and v_0, respectively) and a replication rate (r and r_0, respectively). The decisive contribution to death is the immune response. Thus, the death term is the product of the virus population size (v and v_0), the density of the immune responses (z) and a rate constant (s and s_0) that accounts for the rate of recognition and the elimination of a virus or virus-infected cell by immune responses. Hence, for the growth rate of the two virus populations:

$$dv/dt = (r - sz)v \qquad (1)$$

$$dv_0/dt = (r_0 - s_0z)v_0 \qquad (2)$$

Thus, the mutant population, v_0, can outcompete the wild-type population, v, if:

$$r_0/s_0 > r/s \qquad (3)$$

We call this the 'dominance condition', because it determines whether the attenuated virus dominates the wild-type virus by selection.

The crucial point is that the competition of two variants is determined solely by the ratio of replication rate to immune elimination. Since both variants are exposed to the same immune response, z, selection does not depend on the strength of the immune response. In particular, selection is independent of the viral parameters that determine the stimulation or growth rate of the immune response. So far, this model does not include any virus-specific features, rather it represents a first approximation of the dynamics of two replicating microorganisms in the same host under crossreactive immunological attack.

A simple form that describes the dynamics of the immune response during HIV infection is:

$$dz/dt = kv - k_0v_0 - (uv + u_0v_0)z - bz \qquad (4)$$

Here, we assume that the growth rate of the immune response depends both on the amount of each virus variant (v and v_0, respectively) and a variant-specific stimulation rate (k and k_0, respectively). HIV impairs the immune responses by direct or indirect killing of T helper (Th) cells. This is reflected by the terms $-uvz$ and $-u_0v_0z$. Finally, we include a term $-bz$, which accounts for the natural decay or degradation rate of the immune response.

The attenuated virus can be controlled by the immune response, if:

$$r_0u_0 < k_0s_0 \qquad (5)$$

We call this the 'safety condition', which specifies that the attenuated virus must not cause disease. In the

absence of an infection by the wild-type virus, the attenuated virus will converge to the equilibrium viral load $v_0 = br_0/(k_0 s_0 - r_0 u_0)$. Essentially, a more realistic safety condition should require this equilibrium load to be very low, such that the attenuated virus does not cause any symptoms.

On the other hand, we may assume that the wild-type virus cannot be controlled by the immune response, i.e. that the rate constants fulfil the relationship:

$$ru > ks \qquad (6)$$

In principle, the inequalities described by Eqns 3, 5 and 6 can all be fulfilled simultaneously. Hence, it is possible that, while the wild-type virus causes disease, the attenuated strain may fulfil both the safety and dominance conditions. This means that an attenuated virus variant can outcompete and replace a disease-inducing variant within a patient. Thus, post-exposure administration of an attenuated variant could potentially modify or even prevent disease, if the attenuated variant is competitively superior to (and dominates) the disease-inducing wild type within the host.

The mathematical model also clearly outlines the limitations of using an attenuated strain to outcompete a wild-type strain: if, for example, the functional relationship between the replication rate (r) and the killing rate of Th cells (u), and the elimination rate (s) and the stimulation rate (k) of the immune response, are strictly linear (that is, $r = \alpha u$ and $k = \beta s$), then competitive superiority and pathogenicity are equivalent and intra-host competition always leads to increased

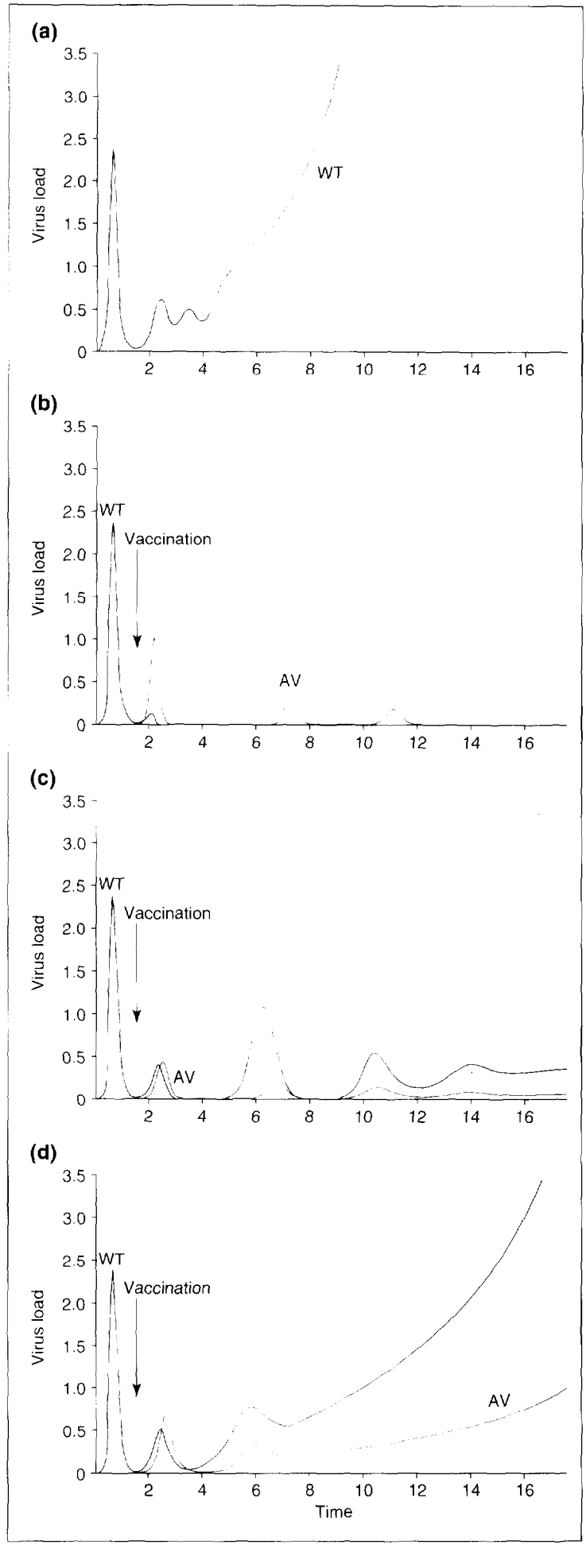

Fig. 2. *A numerical simulation of the differential equations given in Box 1. At equidistant time points, the number of pathogenic variants,* n, *is increased. In a simple way, this models the occurrence of new antigenic escape mutants due to erroneous replication. Time and viral load are shown in arbitrary units. Graph (a) shows the course of disease induced by the pathogenic wild-type (WT) strain. After an initial peak of viraemia, a period of low viral burden ensues in which the immune system is able to control the virus population. Ultimately, the immune responses fail to downregulate the WT virus population and the uncontrolled virus growth results in AIDS. In the three lower graphs, the attenuated virus (AV) vaccine is administered after the initial peak of viraemia (at* t = *1.5). The red line represents the total virus load of the pathogenic WT variants and the green line shows the load of the AV. In graph (b), the AV is able to drive the WT virus population to extinction, and establishes a persistent infection protecting against the development of AIDS. In this parameter region, the WT population cannot coexist with the AV in an individual. Therefore, the AV protects against infection by the WT variants. In graph (c), the AV is not able to eradicate the WT virus, but downregulates the total virus growth such that the immune response retains control over the virus population. This may prevent the development of disease. In graph (d), the AV is no longer able to prevent disease but, nevertheless, delays the onset of disease. Finally, there is a parameter region (not shown here) for which the AV is eradicated by the WT virus population. These are all possible types of behaviour and we have derived a complete mathematical classification of all parameter regions. The parameters chosen for the plots are (in arbitrary units): for WT variants,* $r = 8$, $s = 3$, $c = 3$, $p = 3$, $k = 2.8$, $u = 1.2$ *in all plots; for AV,* $r_0 = 10$, $s_0 = 2$, $c_0 = 3$, $p_0 = 3$, $k_0 = 10$, $u_0 = 1$ *in graph (b);* $r_0 = 9$, $s_0 = 3$, $c_0 = 3$, $p_0 = 3$, $k_0 = 5$, $u_0 = 0.5$ *in graph (c); and* $r_0 = 9$, $s_0 = 3$, $c_0 = 3$, $p_0 = 3$, $k_0 = 4$, $u_0 = 1.2$ *in graph (d).*

pathogenicity. In this case, a nonpathogenic attenuated virus can never protect against disease.

One can also assume that $ru < ks$, i.e. the wild-type virus is also regulated to some equilibrium level. This equilibrium may be higher than for the attenuated strain. It is straightforward to show that a virus strain establishing a high viral load can be outcompeted by a strain establishing a low viral load (Fig. 1). Thus, if pathogenicity is associated with viral load, then a disease-inducing wild-type virus may be outcompeted by a live attenuated virus. Generally, the cause of pathogenesis in HIV infections is a controversial issue, but unless the 'more pathogenic' variant of two competing strains is also always the 'competitively superior' strain then, in theory, the possibility exists that a more pathogenic strain is outcompeted by a less pathogenic competitor.

The model described above was designed to be as simple as possible for outlining the dynamics of post-exposure vaccination. Adding more layers of complexity by no means affects the essentials of our conclusion. We discuss an extended model in Box 1 and Fig. 2, which incorporates strain-specific and crossreactive immune responses, as in the 'diversity threshold' theory of HIV infections[14–17]. The simulations are started with a single pathogenic wild-type virus. New, antigenically distinct, mutant strains arise due to erroneous replication. This is modelled by introducing new pathogenic variants from time to time. Graph (a) in Fig. 2 shows the course of infection by the wild-type variants in the absence of vaccination with the live attenuated strain. AIDS develops when the immune response eventually fails to control the virus growth. Graphs (b), (c) and (d) in Fig. 2 show the possible outcomes of vaccination with an attenuated virus strain after exposure to the same pathogenic wild-type variants as in graph (a). In all cases, the live attenuated vaccine strain is administered shortly after the initial peak of viraemia. The degrees of protection conferred by the attenuated strain range from: full protection against persistent infection by the pathogenic wild-type virus [graph (b)]; protection against disease [graph (c)]; delay of the disease onset [graph (d)]; and no protection at all (not shown). Although Fig. 2 implements the diversity threshold theory as a model for disease progression, the conclusions of this article are independent of any detailed mechanism of pathogenesis (as long as competitive superiority is not directly linked to pathogenicity). Furthermore, it should be emphasized that the mutants described here are not equivalent to defective interfering (DI) particles[18,19], which inhibit viral replication by co-infecting cells together with the wild type. This is not necessary in our model, where the competitive interaction is based on crossreactive immune responses.

Concluding remarks

The key issue of this paper is that competition between different virus variants (in persistent infections) need not be determined by those properties that define pathogenicity. In principle, it is possible that a slow-replicating virus outcompetes a fast-replicating virus and, even more counter-intuitive, that the slow-replicating virus is subsequently reduced to very low concentrations (see Fig. 1). The argument has been outlined using HIV as a specific example; however, it is not restricted either to HIV or to other immune-function impairing viruses.

Co-infection experiments of wild-type and live attenuated virus in a suitable host are necessary to assess the competitive ability of the attenuated virus. There is, of course, no general way of producing such competitively superior, but less pathogenic, mutants; and this seems to be a separate problem for each different virus species. In principle, it may be promising to knockout a gene that is involved in accelerating replication but is also highly immunogenic. This could lead to a mutant with lower r_0 and lower s_0, which may thereby fulfil the dominance condition (Eqn 3). It is possible that the *nef*$^-$ SIV variant[11] already fulfils the essential properties, although preliminary experiments using it as a post-exposure vaccine were unsuccessful (R.C. Desrosier, unpublished). However, the conditions may have been too restrictive in these first experiments: for instance, the vaccinating dose may have been too low, or the administration of the vaccine too late. Perhaps our model can encourage further experiments in these directions.

Our main goal was to show that the basic principles of viral dynamics in the presence of an immune response do not rule out the possibility that slow-replicating attenuated viruses can outcompete fast-replicating pathogenic strains. This may have seemed contrary to expectation before we began.

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Sebastian Bonhoeffer and Martin Nowak are at the Dept of Zoology, University of Oxford, South Parks Road, Oxford, UK OX1 3PS.

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Physiological enzymatic cleavage of leukocyte membrane molecules

Vladimír Bažil

Certain membrane molecules are enzymatically cleaved from the cell surface and then released into the extracellular medium in the form of soluble fragments. This process, commonly initiated by cell stimulation, may regulate the surface expression of such molecules, and may also be responsible for the production of their soluble forms in vivo. *Here, Vladimír Bažil provides an overview of the molecules that are cleaved from cells, focusing particularly on leukocyte receptors. In addition, he discusses the mechanisms and putative enzymes involved in this process, as well as the potential physiological significance of such events.*

Several membrane molecules are cleaved from the surface of leukocytes and other cells by endogenous cellular enzymes, thereby releasing their soluble fragments into the extracellular medium (Table 1). This has been demonstrated *in vitro* using a combination of various methods, including: (1) detection of soluble fragments in the culture supernatant of surface-labeled cells; (2) inhibition of the release of the soluble forms by enzyme inhibitors; and (3) comparison of the apparent molecular weight of the membrane form with the released soluble form.

Two different pathways inducing receptor cleavage

The release of cell-surface molecules following cleavage in the membrane-proximal, extracellular domain is commonly initiated by cell stimulation. Two different pathways inducing this process have been identified. The first of these requires engagement of the relevant membrane protein during the initial stage of cleavage induction. For example, CD14 (Ref. 1), CD43 (Ref. 8), CD44 (Ref. 9) and CD62L (L-selectin)[22,23] are cleaved and released from the surface of leukocytes following incubation *in vitro* with monoclonal antibodies (mAbs) that recognize these individual receptors, possibly simulating the effect of their natural ligands. Crosslinking of the receptors on the cell surface is critical for their effective cleavage. In addition, interleukin 3 (IL-3)[15] and CD27 ligand[24] induce cleavage of their respective receptors, demonstrating that natural ligands are also able to induce this process. The second pathway of receptor cleavage may be initiated either by natural factors which stimulate cells *via* receptors that are different from the molecules to be cleaved, or by phorbol 12-myristate 13-acetate (PMA), a potent synthetic activator of protein kinase C. Thus, cytokines and chemotactic peptides induce cleavage of L-selectin[11,25] and tumor necrosis factor receptors (TNFRs)[14]; immunoglobulins interacting with cell-surface Fc receptors induce cleavage of L-selectin[23]; and anti-CD20 mAbs (Ref. 26) or the CD40 ligand[27] stimulate cleavage of CD23. Furthermore, PMA initiates cleavage of CD14 (Ref. 1), CD16 (Ref. 3), TNFRs (Ref. 14), CD43 (Ref. 8), CD44 (Ref. 9), L-selectin[11,25], IL-6R (Ref. 16), and membrane-anchored precursors of transforming growth factor α (pro-TGF-α), *c-kit* ligand 1 (KL-1) and KL-2 (Ref. 21).

The cleavage-triggering event may initiate two distinct activation processes. One mechanism may include a conformational alteration of the membrane molecule to be cleaved, exposing a cleavage site for the enzyme involved. This process may be induced either by direct binding of a particular ligand, or by a signal transduced from a cytoplasmic compartment that results from activation events independent of the molecule to be cleaved. This intracellular signal may be mediated by the modification of the cytoplasmic tail of the receptor, such as by phosphorylation, or by the association/dissociation of the receptor with other molecules or cytoskeletal components. For example, the C-terminal valine residue located in the cytoplasmic tail of pro-TGF-α has been shown to be essential for PMA-induced cleavage of this membrane-bound cytokine[28]. Thus, an 'inside-out' signaling event that emanates from the cytoplasm, and which requires the pro-TGF-α