Immunological functions of non-professional antigen-presenting cells: new insights from studies of T-cell interactions with keratinocytes

Brian J. Nickoloff and Laurence A. Turka

T-cell activation in the absence of co-stimulatory signals can lead to induction of anergy. Professional antigen-presenting cells (APCs) of bone marrow origin, such as macrophages and dendritic cells, can provide co-stimulation through molecules such as B7-1 and B7-2. In addition, cells of epithelial origin can function as 'non-professional' APCs when activated. In these circumstances, the functional consequences of the T cell–APC interaction may differ, perhaps due to the nature of the co-stimulatory pathways utilized and/or the cytokines encountered by the T cell. Here, Brian Nickoloff and Laurence Turka suggest that these differences may be important in regulating immune responses to local antigens and also in maintaining self-tolerance.

Research accumulated over the past several years has demonstrated convincingly that resting T cells require at least two signals for induction of cytokine gene expression and cell proliferation (reviewed in Refs 1 and 2). Signal 1 can be produced by ligation of the T-cell receptor(TCR)/CD3 complex with antigenic peptides bound to the appropriate major histocompatibility complex (MHC) molecule. Although signal 1 can also be delivered by stimulatory antibodies directed at non-polymorphic portions of the TCR/CD3 complex, it is envisaged that, in physiological situations, signal 1 provides antigen specificity. By contrast, signal 2, often termed the 'co-stimulatory' signal, is not antigen specific and was originally defined as an activity provided by bone-marrow-derived accessory cells such as macrophages or dendritic cells, although activated B cells were also found to be capable of providing co-stimulation. Each of these cells also bears MHC class II molecules and has the capacity to present antigen to $CD4^+$ T cells. The terms 'professional' antigen-presenting cells (APCs) or 'accessory' cells have been coined to describe the population of bone-marrow-derived cells that possess this capability.

The nature of the co-stimulatory signals provided by professional APCs has been the subject of intense scrutiny. Although T helper 2 (Th2)-cell clones can release soluble mediators such as interleukin 1 (IL-1) that may provide signal 2, in most instances it appears that co-stimulatory signals are provided by surface molecules on APCs interacting with their cognate ligands on T cells[1–4]. Several molecules have been provisionally shown to possess co-stimulatory capacity, including heat stable antigen (HSA) (Ref. 5), invariant chain modified by chondroitin sulfate (Ii-CS) (Ref.6) and perhaps intercellular adhesion molecule 1 (ICAM-1) (Ref. 7). However, the co-stimulatory pathway that has received the most attention to date is that mediated *via* the interaction of CD28 on T cells with one of its ligands, B7-1 or B7-2/B70, on professional APCs (Ref. 8). Stimulation of CD28 in this manner leads to induction and enhancement of lymphokine gene expression, a process mediated by intracellular signals distinct from those transduced through the TCR/CD3 complex[9]. CTLA-4, a gene related to CD28, is expressed on T cells and is also a ligand for B7-1 and B7-2/B70 (Ref. 10), although the nature of the signal transduced by CTLA-4 remains unknown[11].

Co-stimulation by non-professional APCs

MHC class II molecules are usually expressed primarily on professional APCs (Ref. 12). However, following cytokine stimulation *in vitro*, or during inflammatory states *in vivo*, MHC class II molecules are induced on a wide variety of epithelial and endothelial cells. Thus, provision of co-stimulatory signals could enable these cells to initiate immune responses in the absence of professional APCs. Experiments using human keratinocytes as an epithelial cell model have provided interesting results in this context[13,14] (Fig. 1). These multi-passaged keratinocytes are prepared from skin biopsies of healthy individuals, and are completely depleted of Langerhans cells (dendritic cells of bone marrow origin that normally reside in the epidermal layer of skin) by multiple passages in tissue culture. Keratinocytes *in vitro* and *in vivo* do not express MHC class II antigens constitutively, but can be induced to do so after exposure to interferon γ (IFN-γ). Following activation, MHC class II-expressing keratinocytes are

able to provide co-stimulatory signals to T cells that have been rigorously purified. These signals are sufficient to support T-cell proliferation following stimulation with bacteria-derived superantigens (SAgs) such as staphylococcal enterotoxin A (SEA) or SEB, phytohemagglutinin (PHA), or anti-CD3 monoclonal antibody (mAb). Thus, MHC class II$^+$ epithelial non-professional APCs are able to function as accessory cells in providing requisite signals for T-cell proliferation (Fig. 1). Similar results have been demonstrated for endothelial cells[15].

The finding that keratinocytes can provide co-stimulatory signals prompted a search to identify the relevant pathway by which this occurs. An obvious candidate was B7, now recognized to be a family of molecules that includes two cloned genes, B7-1 and B7-2/B70 (Refs 16,17). These studies led to the interesting observation that an anti-B7 mAb (now known to recognize B7-1) and anti-BB-1 mAb had disparate patterns of reactivity on keratinocytes. Thus, keratinocytes bound anti-BB-1 mAb but did not react with anti-B7-1 mAb, nor with a CTLA-4–Ig fusion protein that binds both to B7-1 and B7-2 (Ref. 13). The epitope recognized by anti-BB-1 mAb (subsequently termed B7-3) appeared to be a ligand for CD28, based on adhesion studies with CD28 transfectants. However, blocking this interaction with anti-BB-1 mAb does not inhibit keratinocyte-mediated co-stimulation. This suggests that this CD28 ligand might not be mediating co-stimulation in this instance, particularly as anti-BB-1 mAb is an effective inhibitor of CD28-dependent co-stimulation in other systems[14]. On testing a large panel of mAbs, only anti-MHC class II mAbs, and mAbs directed against either lymphocyte function-associated molecule 1 (LFA-1) or ICAM-1, blocked proliferation. In the former case, it can be assumed that inhibition of proliferation is due to prevention of the delivery of signal 1. In the latter case, considerable controversy exists as to whether or not LFA-1 transduces a co-stimulatory signal. When defined as augmentation of proliferation, LFA-1-mediated signals are clearly co-stimulatory[7]. However, when a more-strict definition is used, in which co-stimulation must be active both in *cis* and in *trans*, many investigators do not demonstrate co-stimulatory activity through this pathway[18]. This is also supported by the finding that stimulation through CD28 resulted in the transduction of intracellular signals that are distinct from those elicited by the TCR, whereas LFA-1-mediated signals appear to be qualitatively identical[19,20]. Furthermore, the effect of LFA-1 on proliferation is not necessarily accompanied by co-stimulation of IL-2 production or the ability to prevent induction of anergy[21]. Thus, we favor the interpretation that, in this system, co-stimulatory signals for activated T cells are not being delivered through B7-3–CD28 (based on the inability of anti-BB-1 mAb to block proliferation) or through LFA-1. Instead, alternative co-stimulatory structures are likely to be involved, and the roles of HSA (Ref. 5) and Ii-CS (Ref. 6) are currently under investigation.

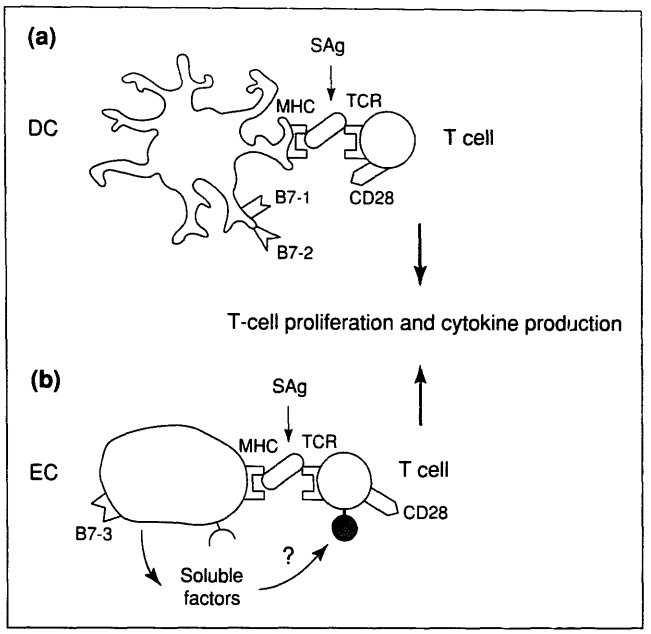

Fig. 1. *Accessory function of professional and non-professional antigen-presenting cells (APCs) such as (a) dendritic cells (DCs) and (b) epithelial cells (ECs), respectively. Bone-marrow-derived professional APCs, as well as non-hematopoietic ECs, can provide co-stimulatory signals to T cells stimulated with superantigen (SAg) via interaction between the major histocompatibility complex (MHC) and the T-cell receptor (TCR). This leads to T-cell proliferation and cytokine production. (a) In the case of professional APCs, the co-stimulatory signals can be delivered when B7 (both B7-1 and B7-2) binds to CD28. Other, as yet uncharacterized, co-stimulatory pathways may also be involved. (b) ECs express neither B7-1 nor B7-2 and deliver their co-stimulatory signals through unknown cell-surface or soluble proteins. Although ECs may express the CD28 ligand B7-3 (BB-1), it is unclear if a CD28–B7-3 interaction provides T-cell co-stimulation. SAgs are shown here as the T-cell stimulus since many ECs do not process proteins into nominal antigens, nor do they stimulate allogeneic T cells (see Fig. 2 and accompanying text).*

The APC type may influence the cytokines produced from resting T cells

Resting T cells have the potential to produce a variety of cytokines upon stimulation, including IL-2, IFN-γ, IL-4, IL-5 and IL-10 (reviewed in Ref. 22). According to one popular theory, these so-called Th0 cells have not yet differentiated into a discrete profile of cytokine production, but do so following continued stimulation. The differentiation into Th1 *versus* Th2 cells is thought to be related primarily to the milieu in which the T cells undergo stimulation, and especially to the cytokines present in the local environment. However, apart from the cytokines the APCs might themselves produce, little is known regarding the potential influence of APCs on this event. Interestingly, whereas co-stimulation *via* CD28 is known to enhance strongly the T-cell production of various cytokines, at least two groups have shown that blockade of this pathway skews T cells towards IL-4 production[23,24]. This suggests that another co-stimulatory pathway may also be available for IL-4 production by naive T cells or by committed Th2-type IL-4-producing cells. Given the fact that non-professional APCs (exemplified in this case by keratinocytes) can provide effective co-stimulation for T-cell proliferation, and that co-stimulation does not appear to be delivered through the CD28–B7 pathway, the question arose as to whether

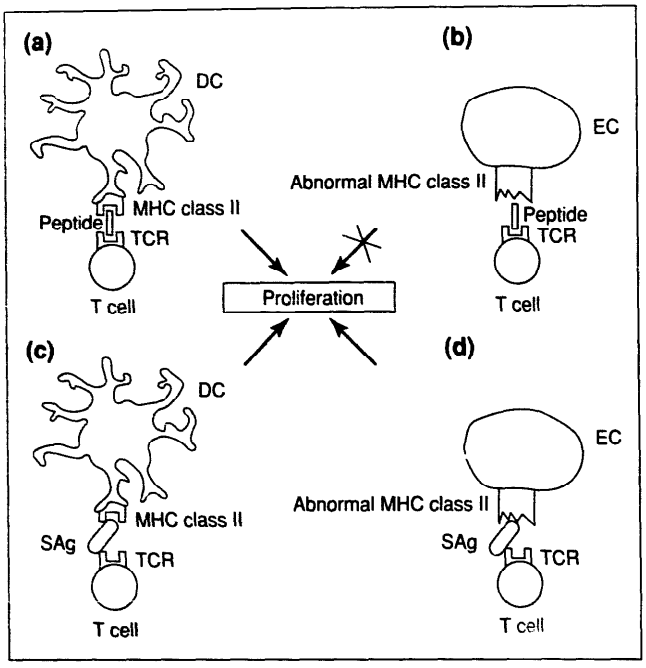

Fig. 2. *Invariant chain (Ii)-negative cells do not stimulate allogeneic T cells. One possible explanation for the inability of major histocompatibility complex (MHC) class II⁻ keratinocytes to stimulate alloreactive T cells is their failure to express normal levels of Ii in the cytoplasm and at the cell surface. Ii is known to affect the conformation and/or peptide-binding ability of MHC class II molecules. (a) T cells bind and respond to allogeneic MHC class II antigens expressed on the surface of Ii⁻ antigen-presenting cells (APCs) such as dendritic cells (DCs). (b) In the absence of normal levels of Ii in epithelial cells (ECs), MHC class II molecules are expressed in an altered conformation (depicted here) and/or with defective peptide binding. These 'altered' MHC molecules may not be capable of stimulating allogeneic T cells via the T-cell receptor (TCR), or there may be a markedly decreased frequency of possible T-cell responders. (c),(d) By contrast, superantigens (SAgs) bind equally effectively to MHC class II molecules from Ii⁻ and Ii⁻ cells, respectively.*

non-professional APCs might lead to a different cytokine profile of stimulated T cells than that seen with professional APCs. Experiments performed to examine this issue showed that, when monocytes were used as accessory cells, a Th0 profile of cytokines (IL-2, IFN-γ, IL-4) was produced[25]. By contrast, when keratinocytes were used as accessory cells, there was a specific defect in IFN-γ production by T cells, while IL-2 and IL-4 responses were intact. Surprisingly however, provision of CD28 activation with a stimulatory anti-CD28 mAb, although augmenting IL-2 production in keratinocyte-supported cultures, did not correct the defect in IFN-γ expression. Mixing experiments demonstrated that keratinocytes could not inhibit the ability of professional APCs to induce IFN-γ production. Together, these data suggest that failure of keratinocytes to induce T-cell production of IFN-γ was not due to 'suppression' or to a lack of productive CD28 stimulation.

Recent work has demonstrated that a key switch that induces Th0 cells to become Th1 cells is the cytokine IL-12 (Ref. 26). IL-12 is known to be produced by macrophages and activated B cells, and to exert an effect directly on T cells. Although IL-12 does not appear to be absolutely necessary to prime cells for IFN-γ production (particularly if IL-2 is present), it greatly augments the amount of IFN-γ produced when compared with cells stimulated in its absence[27]. The ability of IL-12 to prime cells for IFN-γ production is observed even in the presence of IL-4.

Bioactive IL-12 protein comprises two chains of molecular weights 35 kDa and 40 kDa. The 35 kDa chain is constitutively expressed in many cells and is not influenced by cell activation. By contrast, the 40 kDa chain is much more restricted, being expressed in only limited cell types (e.g. professional APCs) and only then after activation. Thus, expression of the 40 kDa chain is ultimately believed to regulate production of the mature protein heterodimer. When keratinocytes were examined by reverse transcriptase polymerase chain reaction (RT-PCR), they were found to express constitutive levels of the 35 kDa chain that were comparable with those observed in peripheral blood mononuclear cells (PBMCs) (Ref. 25). However, the inducible 40 kDa chain was barely detectable in keratinocytes either at rest or following a variety of stimuli, including phorbol ester, tumor necrosis factor (TNF-α) and IFN-γ. Thus, when T cells were stimulated with SAg in the presence of keratinocytes, the 40 kDa chain of IL-12 was present at less than 1/1000th the level of that observed when T cells were similarly stimulated in the presence of professional APCs. Despite this, the overall levels of proliferation and IL-2 production were comparable in both cases. Moreover, when recombinant IL-12 was added to cultures in which keratinocytes were used as accessory cells, two- to tenfold increments in IFN-γ were observed. Together, these data support the concept that the relative lack of IFN-γ production seen in keratinocyte-supported cultures was due to the failure of these cells to produce IL-12.

Mechanisms by which APCs can determine cytokine production

There are at least two possible mechanisms by which APCs can influence the pattern of cytokines produced by T cells, and these are not mutually exclusive. First, and most-directly supported by the data cited above, cytokines produced by the accessory cells providing co-stimulatory signals may be important in dictating the cytokines produced by the T cells. For example, IL-12 greatly augments IFN-γ production, whereas IL-10 tends to block it. Professional APCs and epithelial cells differ in that only the former appear to produce IL-12. By contrast, some non-professional APCs can secrete IL-10 (Ref. 28). Other soluble mediators such as transforming growth factor β (TGF-β) and prostaglandin E_2 (PGE_2), produced to varying degrees by cells capable of delivering co-stimulatory signals, might also be capable of influencing not only the quantity of T-cell-derived lymphokines produced, but also the overall balance amongst various lymphokines.

As mentioned above, a second mechanism by which APCs might dictate qualitative differences in the

production of T-cell cytokines is through the type of co-stimulatory signal delivered. Activation of CD28 is by far the best understood co-stimulatory pathway. However, the newly discovered existence of multiple CD28 ligands[16,17], and the potential influence of signals delivered through the highly homologous CTLA-4 molecule, leave open the possibility that each ligand or pathway might have selective effects on the production of cytokines by T cells. The cytokines induced by other co-stimulatory pathways (e.g. HSA or Ii-CS) have not been studied to a significant extent.

Epithelial cells may not 'present' alloantigen

In several different systems, a variety of investigators have noted that non-professional APCs are poor stimulators of alloreactive T cells in mixed lymphocyte–epithelial-cell reactions. This is often attributed to the inability of epithelial cells to provide co-stimulation. However, as shown above for the case of SAgs, keratinocytes can provide signal 2, yet these same MHC class II-expressing cells fail to induce proliferation of allogeneic T cells. One potential explanation is that allo-stimulated T cells require a co-stimulatory signal distinct from the one provided by keratinocytes. For example, *in vivo* studies have suggested that alloreactions are exquisitely dependent on the interaction of CD28/CTLA-4 with B7-1 or B7-2 (reviewed in Ref. 8). However, even when productive CD28 engagement is provided by a stimulatory mAb, allogeneic T cells fail to proliferate (Ref. 14; L.A. Turka *et al.*, unpublished). Similarly, only a weak response is observed when a keratinocyte cell line is transfected with B7-1 (Ref. 29; B.J. Nickoloff *et al.*, unpublished).

An alternative explanation is that the nature of the peptide–MHC class II complex on keratinocytes differs from that on professional APCs (Fig. 2). Several lines of evidence support this hypothesis. First, in professional APCs, the non-polymorphic class II-associated Ii is intimately involved in processing, transport and peptide presentation of MHC class II molecules[30]. Second, in the absence of Ii, protein antigens are poorly presented: MHC class II molecules made in cells lacking Ii have distinct conformational 'abnormalities', as demonstrated by different reactivities with anti-MHC class II mAbs (Ref. 31). Finally, murine intestinal epithelial cells[31] and human keratinocytes[32] both lack or express only low levels of Ii, and neither cell type stimulates allogeneic T cells. In the latter instance, a low level of proliferation is observed if professional APCs autologous to the T cells are added, most likely a result of indirect presentation of alloantigen. This hypothesis predicts that SAgs are capable of binding to MHC class II molecules synthesized in Ii-deficient cell types. Consistent with this, Ii-knockout mice are capable of negative selection of self-SAg-reactive T cells[33]. A testable prediction of this hypothesis is that transfection of Ii into keratinocytes would reconstitute their ability to stimulate allogeneic T cells.

The epithelial environment

Naturally, there are professional APCs within epithelial surfaces. Clearly, one has to take into account the functional contribution of all potential APC–T-cell interactions to appreciate fully the 'net' immunological outcome. It is not the intention of this article to minimize the importance of professional APCs but to point out that, by their sheer number, epithelial cells such as keratinocytes should not be neglected from consideration. In some instances, external factors may push the immunological balance further towards an epithelial cell 'sphere of influence'. For example, exposure of skin to ultraviolet light leads to a loss of Langerhans cells in the epithelial layer, and induces keratinocytes to produce IL-10 (Ref. 34). Thus, the resulting environment is one in which epithelial cells may drive or predominate in the initial immune response. Even when professional APCs subsequently remigrate into the epithelium, they will encounter T cells that have been stimulated and have begun to differentiate under 'epithelial-dominant' conditions. It will be important to determine if these T cells are irrevocably committed to production of Th2 cytokines, or if this pattern can be subsequently reversed.

Box 1. Epithelial cells in immune responses

Original ideas	Extended concepts
Epithelial cells bearing MHC class I antigens are passive targets for T cells.	Epithelial cells can be active initiators of immune responses, providing co-stimulatory signals to resting T cells. Lack of alloreactivity could be due to intrinsic differences in MHC class II molecules (e.g. diminished invariant chain) rather than from lack of co-stimulatory capacity.
CD28/CTLA-4 ligands are restricted to hematopoietic cells.	Epithelial cells express BB-1/B7-3, an additional CD28/CTLA-4 counter-receptor. The effects of CD28–BB-1 engagement are unknown.
The determinants of whether stimulated T cells differentiate into Th1 or Th2 cells include the duration of stimulation, the nature of the antigen, the cytokine milieu and the genetic background of the animal.	The nature of the APC, professional *versus* non-professional, may play a role in determining whether T cells become Th1 or Th2 cells.
T-cell anergy following interactions with epithelial cells is the result of failure to receive a second signal.	Epithelial cells may induce effective Th2 responses. The absence of a cell-mediated (Th1) response can appear as anergy depending on the assay used.

Abbreviations: MHC, major histocompatibility complex; Th1, T helper 1; APC, antigen-presenting cell.

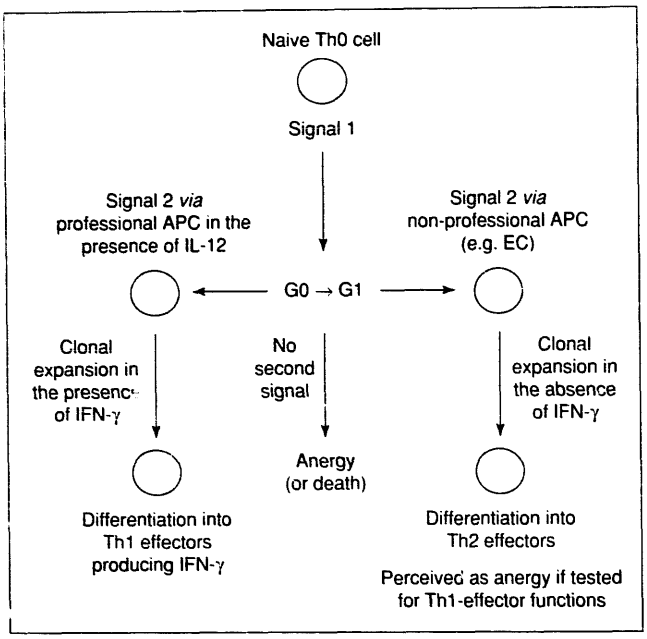

Fig. 3. *Varying consequences of antigen encounter. Resting T cells, so-called T helper 0 (Th0) cells, require at least two signals for induction of cell proliferation and the expression of cytokine genes. Stimulation of the T-cell receptor (TCR) provides signal 1 and, in the absence of a second signal, T-cell anergy (or perhaps death) ensues. Delivery of signal 2 by a professional antigen-presenting cell (APC) is followed by clonal expansion in a manner and environment that favors differentiation into Th1 effectors. Conversely, when signal 2 is delivered by a non-professional APC, such as an epithelial cell (EC), the process favors differentiation into Th2 effectors. If assays of T-cell function are used that depend on the detection of interleukin 2 (IL-2) or interferon γ (IFN-γ) production, or Th1-dependent responses, then this process may be perceived as anergy when, in fact, a specific immune response has been induced.*

Conclusions and implications

Much recent work has extended our understanding of the immune functions of non-hematopoietic cells that are not usually thought of as professional members of the immune system. Endothelial cells are a well-studied example of this, and increasing data indicate that these cells may function as immunocytes. Box 1 outlines some original ideas regarding epithelial cells, and proposes extended concepts based on recent studies. One implication of these ideas (Fig. 3) is that, when epithelial cells induce active T-cell immune responses of the Th2 type, this can either be mistaken for anergy *in vitro* (if assays of Th1 function are only examined) or be functionally equivalent to anergy *in vivo* (if the immune response readout requires Th1 function)[35]. Recent work by Gajewski *et al.*[36] provides support for this concept, demonstrating that 'anergized' Th0 clones may give rise to Th2 cells. In this manner, epithelial cells can regulate the nature of the immune response to local antigens. For example, in the case of skin, failure of keratinocyte-supported responses to induce IFN-γ in T cells, resulting in the the generation of Th2 cytokines, may play a role in peripheral tolerance to self antigens, the induction of IgE-mediated allergic responses (e.g. atopic dermatitis) and the termination of cell-mediated immune reactions (e.g. the challenge phase of allergic contact dermatitis). Disruption of this cytokine balance may be important in autoimmune states such as psoriasis. Conversely, deliberate exploitation of epithelial cells to induce a Th2 response may be effective in inducing transplantation tolerance, and suggests that keratinocytes could be useful cells for gene transfer techniques.

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The suitability of human chorionic gonadotropin (hCG)-based birth-control vaccines

Stephan Dirnhofer, Georg Wick and Peter Berger

It has been widely hoped that immunological methods of fertility regulation by active immunization against specific antigens of the oocyte, sperm, zygote and early embryo, and the placental pregnancy hormone human chorionic gonadotropin (hCG), will provide a means to control the problem of worldwide population growth. The most advanced candidate vaccines are based on hCG immunogens and have entered clinical trials. However, during the past few years, increasing evidence has emerged that the current approaches using hCG as the target molecule may have some major drawbacks. On the basis of their recent findings, Stephan Dirnhofer and colleagues raise doubts on the suitability, safety and efficacy of gonadotropin-based immunological contraceptive vaccines.

The control of worldwide population growth is without doubt one of the most urgent problems of mankind. Global population continues to increase on a hyperexponential scale previously unrecorded in human history, with the result that over 6200 million people will populate our planet by the year 2000. Thus, new and improved methods of fertility regulation are urgently needed and represent a socially compelling and scientifically challenging issue.

In the past years, substantial progress has been made in unravelling the complex interactions between the immune system and the reproductive system, and a number of possibilities for immunological contraception have emerged[1–6]. The rationale underlying research endeavours has been the development of new methods of family planning that provide safe, effective and long-lasting contraception from a single administration that is reversible, inexpensive, practical to use and generally acceptable. The most promising approach uses antifertility vaccines based on, and directed against, molecules essential for the reproductive process. Candidate antigens include those of the sperm membrane, zona pellucida, trophoblast membrane and products, as well as hormones involved in reproductive physiology and, amongst these, human chorionic gonadotropin (hCG) is the forerunner[7–9].

Vaccines against hCG

The hCG hormone is a member of the human (h) glycoprotein hormone family, whose members show close evolutionary, immunological and biochemical similarity. The family also includes luteinizing hormone (LH), follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH). All four hormones