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REVIEW

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Schiff base forming drugs: mechanisms of immune potentiation and therapeutic potential

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Abstract CD4 T-lymphocytes, which orchestrate immune responses, receive a cognitive signal when clonally distributed receptors are occupied by MHC class II bound peptides on antigen-presenting cells. The latter provide costimulatory or accessory signals through macromolecules such as B7.1 and B7.2 which interact with coreceptors on T-cells to regulate outcomes in terms of T-cell activation or specific non-responsiveness. Complementary studies at the chemical level have implicated Schiff base formation between specialised carbonyls and amines, constitutively expressed on antigen-presenting cell and T-cell surfaces, as an essential element in specific T-cell activation. The small xenobiotic Schiff base forming molecule tucaresol, which substitutes for the physiological donor of carbonyl groups to provide a costimulatory signal to CD4 T-helper lymphocytes (Th-cells), has been developed for testing as an immunopotentiatory drug. Tucaresol, which is orally bioavailable and systemically active, enhances CD4 Th-cell and CD8 cytotoxic T-cell responses in vivo and selectively favours a Th1-type profile of cytokine production. In murine models of virus infection and syngeneic tumour growth it has substantial therapeutic activity. Schiff base formation by tucaresol on T-cell surface amines provides a costimulatory signal to the T-cell through a mechanism that activates clofilium-sensitive K^+ and Na^+ transport. The signalling pathway utilised by tucaresol converges with T-cell receptor signalling at the level of MAP kinase, promoting the tyrosyl phosphorylation of ERK2 by MEK (mitogen-activated protein kinase kinase). The Schiff base forming class of immunopotentiatory drug provides the first orally active, mechanism-based immunopotentiatory agents for therapeutic testing. Tucaresol is currently undergoing pilot phase I/II clinical trials as an immunopotentiator in chronic hepatitis B virus infection, HIV infection and malignant melanoma.

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Abbreviations *APC* Antigen-presenting cell · *IFN* Interferon · *IL* Interleukin · *MAPK* Mitogen-activated protein kinase · *S-NHS-biotin* Sulpho-*N*-hydroxysuccinimidobiotin · *TCR* T-cell receptor · *Th-cell* T-helper lymphocyte

Introduction

CD4 T-lymphocytes, which orchestrate immune responses, receive a cognitive signal when clonally distributed receptors are occupied by MHC class II bound peptides on specialised antigen-presenting cells (APC) [1, 2]. APC also provide costimulatory or accessory signals, and it is these second signals that determine the outcome of T-cell receptor (TCR) ligation by antigen in terms of either T-cell activation, leading to an immune response, or to an anergic state in which the immune system can becomes non-responsive to that particular antigen [3]. The interaction between APC and T-cell is therefore a central target for therapeutic strategies aimed at modulating the immune response. In chronic infectious diseases and in cancer it is therapeutically desirable to strengthen the immune response against infectious pathogens or tumours. To this end cytokine therapies with interleukins (IL) 2 [4, 5] and 12 [6, 7] have been pursued, together with gene therapy approaches in which costimulatory molecules such as B7.1 are expressed in tumour cells [8, 9]. Although it is well-recognised that small, orally available, systemically active drugs are ultimately preferable to cumbersome and costly biological therapies, no mechanism-based, orally active immunopotentiatory drugs have been available for clinical testing.

The study of T-cell interaction at the chemical level, complementary to studies at the macromolecular level, has implicated transient covalent chemical events between cell-surface ligands on APC and T-cell as essential in T-cell activation. These take the form of Schiff base

formation between specialised carbonyls and amines [10–14]. The discovery of this chemical mechanism provides a new target for the manipulation of immune responses. Because small Schiff base forming molecules can substitute for the physiological donor of carbonyl groups and provide a costimulatory signal to T-cells, the Schiff base mechanism permits a rational medicinal chemistry approach to the design of orally active immunopotentiatory drugs [15].

Costimulatory mechanisms in the induction and regulation of Th-cell responses

A number of macromolecular interactions between APC and T-cell have been implicated in providing costimulation to $CD4^+$ T helper lymphocytes, and the ligation of the T-cell macromolecule CD28 by accessory cell macromolecules B7.1 and B7.2 are principal examples [16–18]. CD28 in particular has been extensively implicated in the provision of an essential signal whose absence results in T-cell anergy – an important mechanism in the continued maintenance of non-responsiveness to self and other non-harmful antigens [3] (Fig. 1).

The ligation of T-cell CD2 by accessory cell CD48, CD58 and CD59 also appear to be important in providing a costimulatory signal to T-cells [19]. CD2 can substantially enhance TCR dependent responses, producing up to five-fold increases in the production of IL-2 which mediates the expansion of T-cell numbers. Additional enhancement of T-cell responses to antigen are also mediated by other macromolecular interactions, including lymphocyte function-associated antigen/intercellular adhesion molecule 1 and 2 and very late antigen 4/fibronectin [20]. The intracellular signalling mechanisms utilised

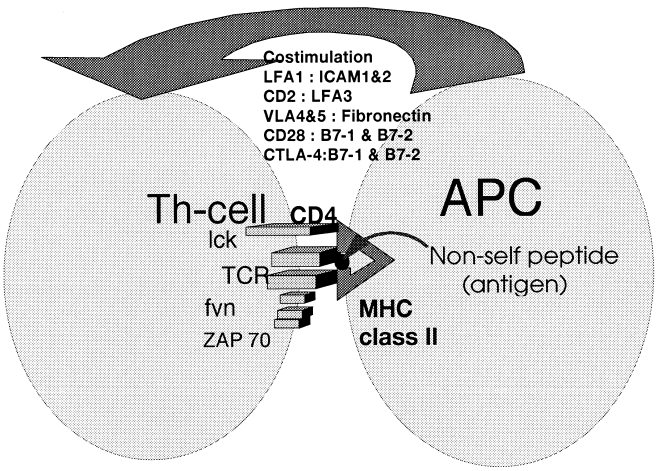

Fig. 1 CD4+ T-cells receive a cognitive signal when clonally distributed receptors for antigen are ligated by peptide antigens bound to MHC class II molecules (signal 1). The outcome of this event is regulated by accessory or costimulatory signals transmitted by macromolecular interactions such as those between B7.1 and CD28. Such signals (signal 2) appear to be essential for T-cell activation

in the costimulatory process are only just beginning to be explored, but it seems that both protein and lipid kinases, which become associated with cell-surface receptors, are involved in transmitting costimulatory signals [21, 22]. The mitogen-activated protein kinase (MAPK) cascade – which activates a family of serine/threonine kinases controlling the activity of transcription factors – has recently been implicated in transmission of signals determining T-cell activation versus anergy [23, 24].

Responses mediated by $CD4^+$ T-helper lymphocytes (Th-cells) can be functionally divided into two types, termed Th1 and Th2 [25, 26]. Th1-type responses produce a profile of cytokine production characterised predominantly by IL-2, γ-interferon (IFN) and IL-12 production. This type of response favours the induction of delayed-type hypersensitivity and cytotoxic T-cells and mediates the elimination of intracellular parasites such as viruses, mycobacteria and protozoa, as well as immunogenic tumour cells. Th-2 responses are characterised by cytokines such as IL-4, IL-5, IL-6 and IL10. In this case the response is biased towards humoral immunity. T-cells also play a crucial regulatory role in limiting and terminating immune responses whose job has been completed, and this is evident in the cross-regulation of immune reponses between Th1 and Th2 compartments [27]. Thus, γIFN blocks many of the effects of IL-4, including Ab class switching and Ab secretion that characterises the maturation of the humoral response. IL-12 also exerts an inhibitory influence on Th-2 type responses. Conversely, IL-4 and IL-10 act as inhibitors of γIFN production and function. In this way the nature of an immune responses may be re-directed as it matures, and this is part of the process that limits unwanted elements of immunity such as continuing inflammation and tissue damage following the elimination of a virus infection. Costimulatory signals appear to have a role in regulating the direction of Th-cell responses, and there is some evidence that B7.1-induced signalling may be more prominent in Th-1 responses, whereas B7.2 -induced signalling may have a more prominent role in Th-2 type responses [28].

The effects of Schiff base forming drugs in T-cell costimulation and regulation

Complementary studies at the chemical level have shown that as a consequence of some of these macromolecular interactions covalent chemical reactions occur between constitutive carbonyl groups and amino groups expressed reciprocally on APC and T-cell surfaces [10–14]. The initial evidence relied on (a) chemical modifications of cell-surface Schiff base forming ligands to block inductive APC:T-cell interactions, (b) re-donation of Schiff base forming groups by oxidising chemicals and enzymes to restore APC:T-cell interactions, (c) the use of the selective reducing agent cyanoborohydride to label Schiff bases and render them irreversible, and (d) cell surface labelling techniques to visualise constitutive Schiff base forming ligands. Figure 2 is a schematic dia-

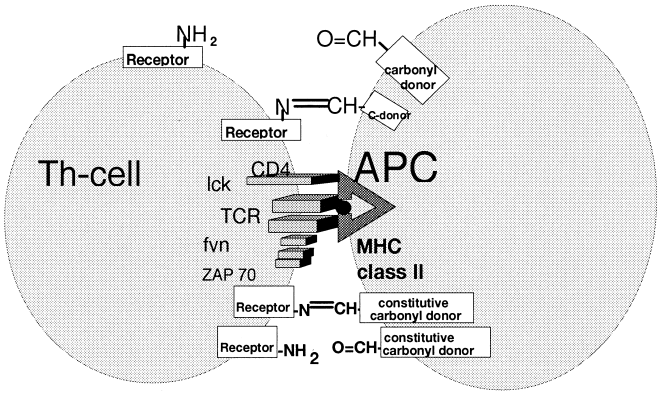

Fig. 2 As a consequence of certain macromolecular interactions, transient covalent chemical events in the form of Schiff base formation between specialised carbonyls and amines take place, and this process appears to be essential for optimal T-cell activation. This reaction is readily reversible [29]. These chemical events provide a target for the manipulation of immune responses

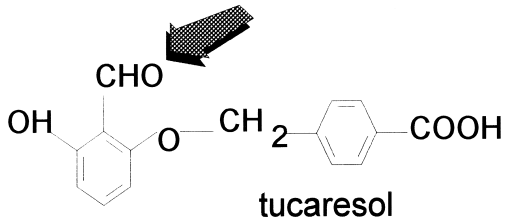

Fig. 3 Stucture of tucaresol. *Arrow*, cell-surface amines react with carbonyl group

gram of these events. Schiff base formation is a readily reversible covalent reaction at physiological pH with the products and reactants tending to an equilibrium [29]. However, Schiff bases can be rendered irreversible by reduction with cyanoborohydride. The importance of Schiff base formation as a physiological process in APC:T-cell interaction provides an explanation for the earlier experimental phenomenon of oxidative mitogenesis in which artificial generation of cell surface Schiff base forming groups had been shown to be a potent mitogenic stimulus for T-cells in vitro [30].

Schiff base formation occurring between constitutive ligands during APC: T-cell inductive interaction appears to be an important element in the costimulatory mechanism. Small exogenous Schiff base forming molecules, some of which are xenobiotic, provide research tools to examine the effects of this covalent chemical event. The substituted benzaldehyde tucaresol, the leading molecule in this class, has been the most studied [15].

Tucaresol (and other Schiff base forming agents) enhances antigen-specific T-cell proliferative responses when added to cultures containing APC and T-cells. Enhancement is optimal when the Schiff base forming molecule is added 24 h before the antigen. This may be because the compound acting simultaneously with TCR ligation may interfere with Schiff base formation occurring between constitutive ligands. The latter may be important both in costimulatory signalling and in mediating the adhesive interaction between APC and T-cell. Tucaresol is also potent in potentiating immune responses in vivo [15]. Used as an adjuvant (i.e. mixed with a vaccine antigen and administered subcutaneously) tucaresol and related compounds potently enhance antigen-specific T-cell responses occurring in regional lymph nodes, and in this respect are comparable to aggressive physicochemical adjuvants.

The formation of a Schiff base by tucaresol on T-cell surface amines can be visualised by competitive covalent ligation of surface amines with sulpho-*N*-hydroxysuccinimidobiotin (S-NHS-biotin). Cell-surface amines react with the carbonyl group of tucaresol (arrow, Fig. 3), and the resultant Schiff base is subsequently rendered irreversible with the selective reducing agent cyanoborohydride. This reaction is then measured by the loss of free cell-surface amino groups reacting with S-NHS-biotin [15]. The latter is measured with fluorescent avidin labelling and flow cytofluorometric analysis. Schiff base formation by tucaresol on cell surface amines, which proceeds within seconds, can also be measured by spectrophotometric methods and by radiolabelling with cyanoborotritiide or radiolabelled drug. In the absence of any TCR-directed stimulus tucaresol has no effect on cytokine production, nor does it produce a proliferative response at any concentration. However, TCR-induced responses are substantially enhanced by tucaresol. Optimally, a five- to ten-fold enhancement of IL-2 production is observed when the T-cell is stimulated with anti-CD3 (mimicking TCR ligation by antigen). Tucaresol favours a Th1-type profile of cytokine production, enhancing the production of IL-2 and γINF [15]. In contrast, the production of the Th2 cytokine IL-4 is reduced in the presence of tucaresol while IL-6 is unaffected [15]. As discussed above, this is likely to be favourable in immunopotentiatory therapy for intracellular pathogens such as viruses, mycobacteria, and protozoal parasites, as well as immunogenic tumours.

These effects in vitro are fully reflected in vivo, and indeed the drug is considerably more potent in vivo where low concentrations produce very substantial effects in the presence of antigen. This may be because of the drugs susceptibility to oxidation in aqueous solutions in vitro. A consistent feature of immunopotentiation by tucaresol is the bell-shaped nature of the dose-response curve. While 200- to 300-μM concentrations are optimal in vitro, the immunopotentiatory effect is lost above 600 μM. In mice, daily oral doses of 20 mg/kg are optimal while the immunopotentiatory effect has declined substantially at 50 mg/kg. Tucaresol given parenterally or orally at an optimum dose potently enhances both the $CD4^+$ Th-cell response to administered antigens and the generation of $CD8^+$ cytotoxic T-cells against viral antigens [15]. In the latter study mice were immunised with a hybrid peptide containing immunodominant helper and a cytotoxic T-cell epitopes of the nucleoprotein of human influenza A [14]. Because both helper and

cytotoxic T-cell epitopes were provided, we do not yet know whether tucaresol exerts direct effects as a costimulator on $CD8^+$ T-cells. Tucaresol is therapeutically effective in murine models of virus infection and tumour growth. In an acute model of cytomegalovirus infection tucaresol substantially reduced viral load over a 5-day course of treatment. In a model of syngeneic tumour growth tucaresol given every 2nd day over a 14-day period reduced the outgrowth of the weakly immunogenic adenocarcinoma MCA38 by 70% [15]. Consistent with a T-cell dependent mechanism, no significant effects on tumour growth were seen in T-cell deficient (athymic) mice.

Mechanisms of Schiff base signalling in physiological systems

In order to begin to understand the mechanism of Schiff base signalling in immune induction and its potential therapeutic exploitability, we need to consider physiological precedents in which a good deal is already known about mechanisms of Schiff base-mediated signalling. In chemical terms, Schiff base formation is a carbonyl-amino condensation that occurs as a result of nucleophilic attack by the amine on the carbonyl group in the following reaction:

$$R_1—C{=}O + NH_2—R_2 \rightarrow R_1—C{=}N—R_2 + H_2O$$

The term is properly applied to the reaction when the nitrogen of the amine is attached to carbon. If it is attached to another nitrogen, the condensation product is a hydrazone rather than a Schiff base. If the carbonyl compound is an aldehyde, the resulting Schiff base is an aldimine; if a ketone, it is a ketimine. The extent of imine formation is markedly dependent of the structure of the carbonyl compound and of the amine. Aldehydes react more rapidly and more completely than do ketones of similar structure. The condensation reaction is believed to be general acid catalysed, and the initial step is the nucleophilic attack by the amino nitrogen on the electron deficient carbonyl group to form the aminocarbinol. Such aminocarbinols are usually unstable and either revert to the starting materials or dehydrate to the imine. This reaction is readily reversible and in consequence the imine exists in aqueous solution as an equilibrium with the aminocarbinol, the carbonyl compound and the amine. In general terms the imine or Schiff base product is itself a a reactive species and is prone to further reactions resulting in the addition of nucleophilic agents (e.g. H_2NR, HSR and HOR) to the imine bond [29].

Schiff base formation is essential in a number of dynamic physiological processes. These include enzyme-substrate interactions such as those in transamination, decarboxylation, and other amino acid modifying reactions mediated by pyridoxal phosphate [31]. Other examples of covalent catalysis include muscle aldolase, lysyl oxidase and acetoacetate decarboxylase [32]. In all cases where Schiff base formation is important in a physiological process the carbonyl donor is an organic prosthetic group or coenzyme such as pyridoxal phosphate. In the case of lysyl oxidase the carbonyl donor is the di-ketone moiety of pyrroloquinoline quinone [33].

Schiff base formation is also central to a number of signal transduction processes. The visual system provides an important example. Schiff base formation between retinal (vitamin A aldehyde) and rhodopsin is central to the process of changing light energy into a neural signal in the form of Na^+ flux [34]. Light falling on 11-*cis*-retinal produces a conformational change which results in a 3-Å shift in the Schiff base linkage in relation to the ring structure of the chromophore. This produces a conformational change in rhodopsin which continues with the transition of retinal through a series of intermediate isomeric forms until the deprotonated all-*trans* form which dissociates and diffuses away because it does not fit the 11-*cis* binding pocket of rhodopsin. This conformational change in rhodopsin produces photoexcited rhodopsin which activates a G protein, transducin which in turn links the activation event to a phosphodiesterase which hydrolyses cyclic GMP. The fall in cGMP levels then *closes* cation specific ion channels in the rod plasma membrane. In the dark, sodium ions flow rapidly into the outer segment of the rod because the electrochemical gradient, maintained by the Na^+ K^+ ATPase pumps located on the inner segments, is large. The closure of these Na^+ channels via light-activated rhodopsin inhibits Na^+ influx resulting in hyperpolarisation. Schiff base formation by tucaresol on nucleophilic amines on the plasma membrane of T-cells has marked effects on Na^+ and K^+ flux (see below) providing an interesting parallel with the visual system. Schiff base formation is also at the centre of light-driven ion pumps in bacteria. This is an intriguing field currently providing some of the most interesting observations in receptor structure:function relationships [35]. The pumps are seven *trans*-membrane (rhodopsin-like) receptors, and their chromophores consist of retinal linked to a lysine residue be means of a protonated Schiff base near the middle of helix G.

The sequence similarity of the proton pump bacteriorhodopsin and the chloride pump halorhodopsin has led to speculation that the mechanism of ion transportation might be similar even though one translocates a cation and the other an anion in opposite directions. In bacteriorhodopsin, proton transfer from the retinal Schiff base to aspartate-85 is crucial in the transport cycle. Remarkably, it has recently been shown that substituting the bacteriorhodopsin aspartate-85 for the halorhodopsin threonine-85 functionally converts bacteriorhodopsin into halorhodopsin when expressed in *Halobacterium salinarium* [36]. Although we are at an early stage in exploring details of the mechanism in immune induction, these ancient and widely distributed light-driven mechanisms may eventually have something to teach us about events in the immune system.

Mechanism of action of immunopotentiatory Schiff base forming drugs

How does the formation of a Schiff base by small exogenous molecules on specialised T-cell surface amines provide a costimulatory signal to the T-cell? Electron probe X-ray microanalysis studies have shown that the formation of a Schiff base on T-cell surface amines produces marked changes in the levels of intracellular potassium and sodium [15]. The resting state of high potassium/low sodium is reversed by tucaresol within 5 min of administration of the drug. By 2 h levels have reverted to the resting state. These large changes are similar to those occurring, for example, in epithelial cells stimulated with epidermal growth factor. The tucaresol-induced changes in Na and K are prevented by ligation of cell surface amines with S-NHS-biotin before the addition of Schiff base forming drug. The changes are also completely prevented by low concentrations of the potassium channel antagonist clofilium tosylate. We know that these changes are an essential element in the immunopotentiatory effects of tucaresol because clofilium likewise inhibits the costimulatory effects of tucaresol in terms of enhancement of IL-2 production by T-cells (Fig. 4).

Convergence of tucaresol costimulation with the TCR-dependent pathway has been identified at the level of the MAPK ERK2 [37]. MAPK is a serine/threonine family of kinases activated by tyrosyl phosphorylation and important in the downstream transmission and integration of a wide range of mitogenic and activational extracellular signals [38]. Preliminary studies in the Jurkat J6 T-cell line, show that stimulation via the TCR with anti-CD3 induces the phosphorylation of MAPK, and so too does stimulation with tucaresol. When both stimuli are given together the phosphorylation of MAPK is substantially enhanced and prolonged. A selective synthetic inhibitor of MAPK tyrosyl phosphorylation, which acts at the level of the kinase one step upstream of MAPK (MEK, a MAPKK) [39], prevents both the phosphorylation induced by tucaresol and the costimulatory effects of tucaresol in terms of enhancement of IL-2 production in response to TCR-CD3 stimulation.

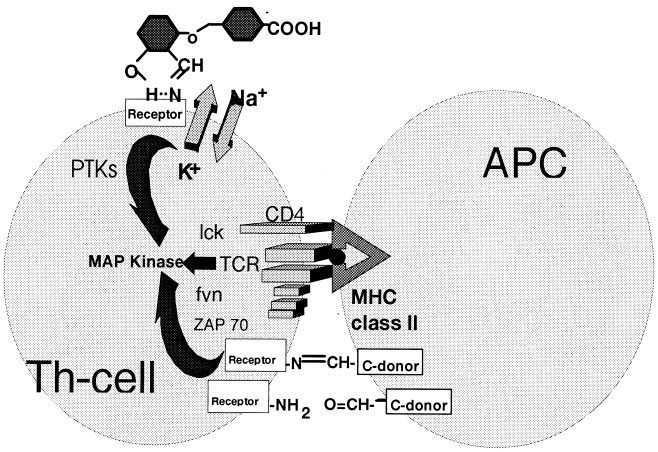

Fig. 4 The formation of a Schiff base by tucaresol on specialised T-cell surface amines provides a costimulatory signal to the T-cell through a mechanism that activates sodium and potassium transport. Tucaresol mediated costimulation converges with TCR signalling at the level of tyrosyl phosphorylation of the MAP kinase ERK2

While some of the important events in the costimulatory pathway exploited by tucaresol have been identified and are in the process of being characterised, other equally important questions remain unanswered. Amongst these are the identity of the cell-surface macromolecules that transduce a costimulatory signal in response to Schiff base formation on functionally sensitive amino groups. What kind of macromolecular process do we envisage taking place? The physiological precedents where Schiff base formation in at the centre of a signal transduction process would suggest a mechanism in which Schiff base formation takes place on conformationally pivotal lysyl ε-amino groups expressed in the ligand binding site of integral membrane proteins. Such an event could induce a conformational change in the macromolecule thereby transmitting a signal across the plasma membrane. One of the initial observations on Schiff base formation in immune induction showed that E-rosette formation at room temperature provided an analogue for Schiff base formation in APC:T-cell inductive interaction [12]. This cellular interaction reflects macromolecular interactions between CD2 and its ligands, especially lymphocyte function-associated antigen-3. These observations together with the markedly similar profile of CD2 costimulation and tucaresol costimulation, make CD2 an attractive candidate as a principal receptor for Schiff base costimulation. [40, 41]. The recently described three-dimensional structure of glycosylated human CD2 reveals certain structural features that we might expect of a receptor for Schiff base costimulation [42]. The ymphocyte function-associated antigen-3 binding domain contains an unusual concentration of lysyl residues whose ε-amino groups appear to be conformationally pivotal. The unusual concentration of positive charge generated by these groups is destabilising to such a degree that it is the function of a mannose-rich N-linked glycan to counter this effect and maintain stability. In the absence of this check the protein unfolds. These groups are thus poised to generate macromolecular conformational change – just the feature to be expected of a target for Schiff base signalling across the plasma membrane. We know by direct labelling and immunoprecipitation, that tucaresol reacts with CD2 and are seeking to ask, by a number of techniques, whether tucaresol initiates a signal through CD2.

Another intriguing question is the nature of the natural donor of carbonyl groups mimicked by costimulatory Schiff base forming drugs. In all cases where Schiff base formation is important in a physiological process the carbonyl donor is an organic prosthetic group. The coenzyme pyridoxal phosphate is an important example [31] while pyrroloquinoline quinone fulfils this role in the enzyme lysyl oxidase [33]. The role of retinaldehyde in

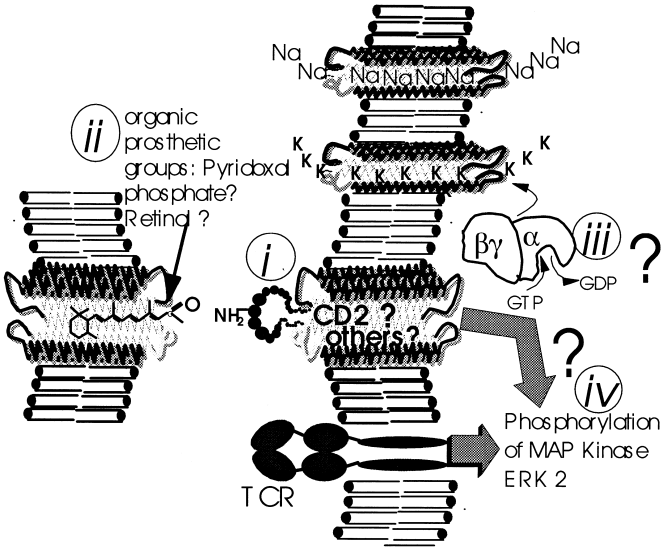

Fig. 5 Possible macromolecular mechanisms in Schiff base costimulation. *i*, Macromolecules receiving Schiff base costimulation. CD2 is an attractive candidate as a principal receptor for Schiff base costimulation. *ii*, Identity of natural donor of carbonyl groups. Many physiological precedents suggest that the natural donor is likely to be an organic prosthetic group such as retinal or pyridoxal phosphate. *iii*, The early events linking signal transduction to ion transport and tyrosyl phosphorylation have yet to be characterised. *iv*, The upstream elements in the MAPK cascade utilised by Schiff base costimulation have yet to be characterised

light-driven signal transduction has already been described [34]. Vitamin A appears to play an essential role in the immune system [43–45], and it is incumbent upon us as immunologists eventually to identify a specific locus of action. At the same time in APC:T-cell interaction we have to identify a physiological donor of Schiff base forming carbonyl groups, a function in which retinaldehyde, in other physiological systems, is a principal player. Of course there may be several loci of action for vitamin A in immunity, and one that does not appear to involve retinaldehyde is in the process of being elucidated [46, 47]. Pyridoxal phosphate is also an interesting candidate in the light of recent evidence suggesting a role for its precursor, vitamin B_6, in T-cell-mediated immune responses [48,49]. We are currently employing a number of techniques to address these questions. Schiff base forming prosthetic groups are likely to be light-sensitive, and this could potentially explain some of the photolabile features of the immune response in terms of immunosuppression and anergy induced by UV light treatment in vivo and in vitro [50–52] (Fig. 5).

Significance of the Schiff base mechanism in immunopathology

The importance of the Schiff base mechanism in immune activation illuminates an entirely new area in immunopathology. In particular the observation that aldehydes and ketones can potentiate immune reponses raises the likelihood that naturally occurring carbonyl-amino reactants may underlie pathogenic processes in autoimmune disease and autoimmunotoxicity. There are a number of intriguing possibilities here, some of which are being actively investigated. For example, bacterial abscess formation induced by amines on capsular polysaccharide (e.g. of *Bacteroides fragilis*) may be due to aberrant signalling initiated by the reaction of these groups with functionally important cell-surface carbonyls of lymphocytes [53, 54]. This possibility is currently being investigated by A. Tzianabos and colleagues at Harvard Medical School. Schiff base forming ketones and aldehydes are generated in the inflamed synovium in rheumatoid arthritis and may well exert immunomodulatory effects that contribute to the disease process [55]. This is a focus of interest for D. Blake and colleagues at the Bone and Joint Research Unit of The London Hospital. The Schiff base mechanism also provides a direct mechanism for the induction of immune damage in alcoholic hepatitis [56]. Acetaldehyde, the primary metabolite of ethanol, is a potent immunopotentiator in vitro and in vivo by virtue of its Schiff base forming reactivity [15]. We tested acetaldehyde in this context, at the suggestion of A. Eddleston of Kings College School of Medicine and Dentistry, London. A fourth area of interest has been generated by the studies of S. Page and colleagues on outbreaks of serious food-related autoimmunotoxicity [57, 58]. Reactive species such as the Schiff base 1,1′-ethylidenebis(L-tryptophan) decomposes to a very reactive carbinolamine, raising the possibility that direct effects of lymphoid receptors sensitive to Schiff base forming molecules may be involved in autoimmunotoxicity.

Potential clinical applications of immunopotentiatory Schiff base forming drugs

It is the function of the immune system to distinguish between normal components of self and harmful foreign or otherwise pathogenic neo-elements, usually, but not exclusively, infectious pathogens. In this way the adaptive anamnestic immune response combats infection, protects against re-infection and may play a role in controlling the emergence of certain tumours, particularly those that give evidence of being immunogenic. An orally active, mechanism-based drug that acts systemically to potentiate the immune system and biases immunity towards the cell-mediated Th1 type of response should have potential applications in a wide range of chronic infectious diseases caused by intracellular pathogens. These include viral infections such as chronic viral hepatitis, HIV infection, mycobacterial infections such as tuberculosis and leprosy, protozoal parasitic infections such as malaria and leishmaniasis, and other parasitic diseases where cell-mediated immunity is likely to be important. As an immunopotentiator, tucaresol is also likely to be complementary to vaccine therapy in which the aim is to provoke cell-mediated or Th-1 type immunity to pathogenic antigens. Such vaccine therapy strategies are currently

being developed for the treatment of chronic hepatitis B virus infection, HIV infection and malignant melanoma using a variety of subunit immunogens designed to stimulate T-helper and cytotoxic T-cell responses together with suitable local physicochemical adjuvants [59–61]. Because tucaresol is an orally administered, systemically active immunopotentiator with an antigen-dependent costimulatory mode of action, it is likely to be effective in enhancing the immune response to administered vaccine antigens as well as antigen presented to the immune system in the normal course of an infection. Oral administration during vaccine therapy would provide a unique means of strengthening the desired immune response and this would not preclude the use of local adjuvants (unlike tucaresol, adjuvants, by their nature, cannot be used systemically). Alternatively tucaresol could potentially be used locally as an adjuvant in vaccine therapy with or without additional oral administration.

Costimulatory Schiff base forming drugs exemplified by tucaresol provide the first mechanism-based orally active immunopotentiatory therapy for clinical testing. The fact that this strategy is entirely new means that it is untested against disease targets. However, pilot clinical studies have been initiated in chronic hepatitis B virus infection, HIV infection, and in malignant melanoma. Convincing evidence of clinical efficacy can only come from the results of carefully controlled clinical trials in potential disease indications where both disease parameters and parameters of immune responsiveness can readily be measured and quantitated. Responses in phase I/II clinical trials will be measured both in terms of disease parameters and T-cell responsiveness to disease specific and nonspecific antigen, anti-CD3 and mitogen. Read-outs will include proliferative responses and production of Th1 and Th2-type cytokines. The aim of immunopotentiatory therapy is to tip the balance of the contest between chronic infection and immunity in favour of the immune system with the minimum effective dose of the drug. With the proper assessment of risk versus benefit and appropriate choice of therapeutic regimen, costimulatory Schiff base forming drugs provide a new opportunity for testing an orally active mechanism-based immunopotentiatory therapy in chronic infectious diseases and cancer.

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