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Review Article

Immunotherapy in head and neck cancer: current practice and future possibilities

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Abstract

The survival of patients with head and neck squamous cell carcinoma has changed little over the last 30 years. However, with recent advances in the fields of cellular and molecular immunology, there is renewed optimism with regards to the development of novel methods of early diagnosis, prognosis estimation and treatment improvement for patients with head and neck squamous cell carcinoma. Here, we present a critical review of the recent advances in tumour immunology, and of the current efforts to apply new immunotherapeutic techniques in the treatment of head and neck squamous cell carcinoma.

Key words: Head and Neck Neoplasms; Squamous Carcinoma; Immunotherapy

Introduction

Head and neck cancer is the sixth most common cancer worldwide, and squamous cell carcinoma accounts for the vast majority of these tumours.[1] While progress in surgical treatment, chemotherapy and radiotherapy has had a significant impact on the outcome of a number of other cancers, there has been little change in head and neck squamous cell carcinoma (SCC) prognosis over the past three decades.[2]

Few non-specialist clinicians have appreciated the potential application of tumour immunology in the management of head and neck SCC. This review discusses the current status of tumour immunology applicable to head and neck SCC and the recent development of novel treatments. The review also highlights some of the remaining obstacles that need to be overcome before any new therapies become readily available to patients.

Tumour immunology

The host immunological response to tumour is similar to an immunological response against an infective organism such as a bacteria or virus. However, because most cancer cells are essentially normal cells that will not stop replicating, it is extremely difficult for the immune system to attack and destroy them effectively, as such self-reactivity is efficiently removed during immune cell production, or actively down-regulated.[3–5]

It is well known that three distinct cell types are mainly responsible for mounting a specific immune response: lymphocytes, natural killer cells and antigen-presenting cells. The roles of these and other components of the immune system have been recently reviewed by us in relation to head and neck SCC.[6] The key interactions are summarised diagrammatically in Figure 1.

Lymphocytes

The effect of proinflammatory T helper-1 cell cytokines (such as interleukin-12 (IL-12) and interferon γ) and anti-inflammatory T helper-2 cell cytokines (i.e. IL-4 and IL-10) have been evaluated in many *in vitro* investigations as well as a gradually increasing number of clinical studies on human head and neck SCC.[7–11] It is generally accepted that relatively high levels of T helper-1 cell type cytokines have both a direct anti-cancer effect and a stimulatory effect on cytotoxic cluster of differentiation 8 (CD8) positive T cells and macrophages. Hence, a T helper-1 cell type response is considered advantageous for solid tumours such as head and neck SCC.[12,13] The T helper-1 and T helper-2 cell cytokines act antagonistically; thus, promotion of a T helper-1 cell response causes down-regulation of the T helper-2 cell response, and vice versa (Figure 2). Generally, in patients with head and neck SCC (and in a number of other malignancies), there is a predominant T helper-2 cell response and a diminished T helper-1 cell response. Whether this is caused directly by a factor released by the cancer or as an indirect effect of the malignancy remains unclear.

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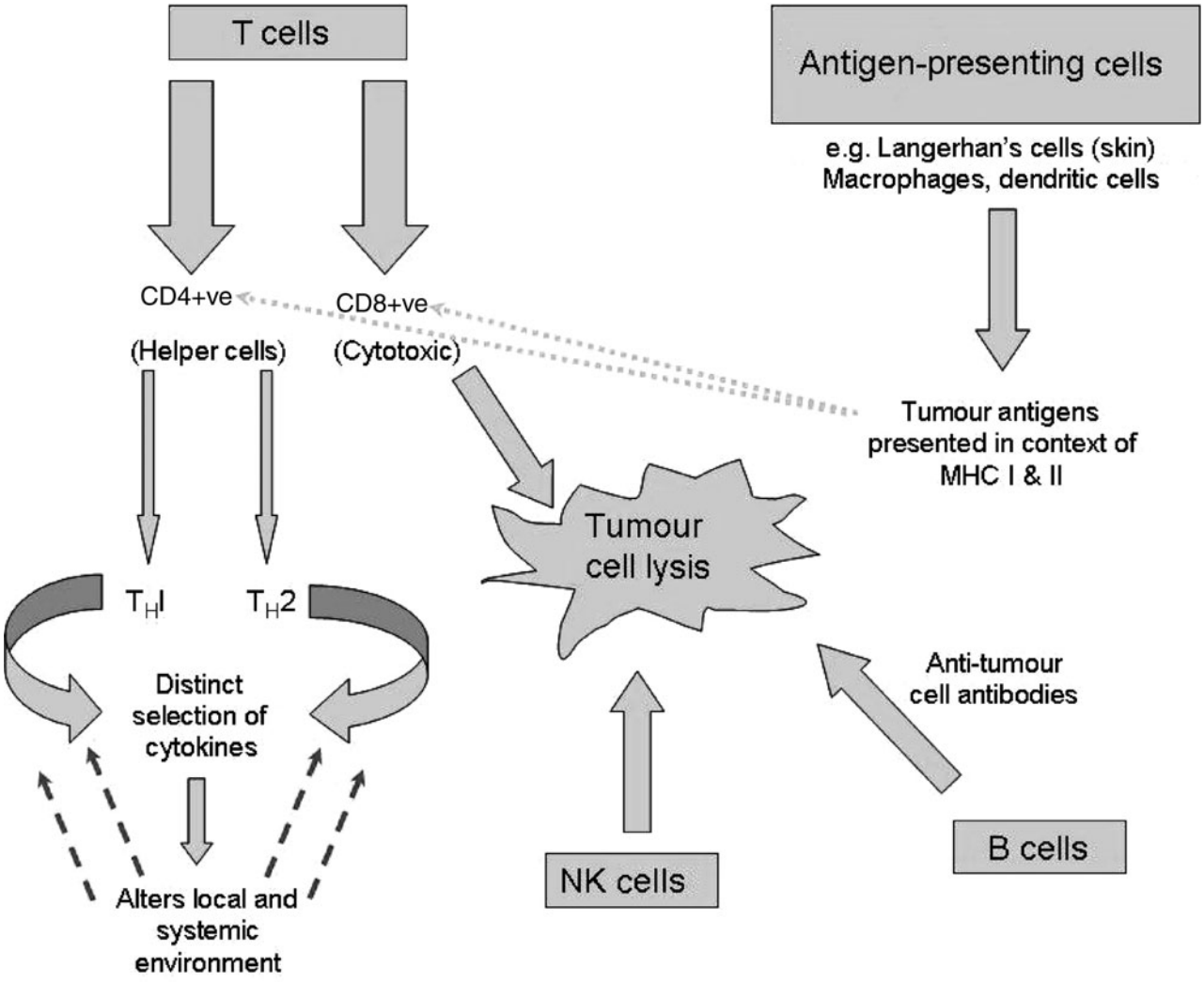

IMMUNE SYSTEM: KEY INTERACTIONS

T lymphocytes can be subdivided into CD8 +ve (cytotoxic) and CD4 +ve (helper) cells, based on expression of the relevant cell surface molecule

CD8 +ve T cells lyse tumours expressing aberrant self peptides bound to host MHC I molecules, *in vitro & in vivo*

CD8 +ve T cells lysis is significantly affected by cytokine environment, either positively or negatively

CD4 +ve T cells principally produce cytokines following activation after recognition of aberrant antigens bound to self MHC II

Both CD8 +ve & CD4 +ve T cells are 'primed' and stimulated by antigen-presenting cells expressing tumour antigens in the context of self MHC, together with essential co-stimulatory molecules

NK cells directly lyse tumour cells that have lower levels or missing MHC I; the cytokine environment will affect performance

B cells produce antibodies against aberrant tumour-associated antigens

Complement cascade thought to play a minor role in tumour cell lysis due to protective, complement inhibitory proteins expressed on cell surface

FIG. 1

Anti-tumour activity of the immune system. CD = cluster of differentiation; +ve = positive; MHC = major histocompatability complex; T_H1 = T helper-1 cell; T_H2 = T helper-2 cell; NK = natural killer

In addition, it is important to note that B cells can contribute to the overall anti-tumour response, through the production of specific antibodies that bind to tumour cells. When combating a bacterial or other extracellular micro-organism infection, highly specific antibodies are produced which bind to the foreign surface and cause effective complement deposition. This subsequently results in the formation of a complex that punctures the cell membrane, as well as the recruitment of immune effector cells such as neutrophils and macrophages. Although this response is powerful and generally highly effective against bacterial infections, the process is generally poor against tumours, even though the cytokine environment is

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TABLE I

EXAMPLES OF CLINICAL TRIALS WITH IMMUNOTHERAPY MODALITIES RELEVANT TO HNSCC (2000–2006)

Study, institute & nature of trial	Trial	Site & stage of tumour	Outcome
Strome[72] UMSM Phase I	MAGE-A3/HPV 16 vaccine for HNSCC (Peptide-based immunotherapy)	Biopsy-proven HNSCC, with progression, or recurrent or metastatic disease	Aims to determine toxicity, changes in anti-HNSCC T cell number & survival
Moyer[73] UMH Phase II	Effectiveness of IRX-2 (product that contains multiple cytokines) in treating patients with locally advanced, operable HNSCC with cyclophosphamide, indomethacin & zinc	Patients with no prior surgery or radiation or chemotherapy Expected to recruit 25 patients	Aims to determine dose & clinical outcome
Timar *et al.*[70] NIO Phase II	Neoadjuvant LI injection of oral carcinoma	$T_{2/3}$ $N_{0/2}$ M_0 oral SCC 19 LI-treated patients & 20 controls	Marked increased & altered composition of tumour-infiltrating mononuclear cells, & increased CD4 + ve: CD8 + ve ratio, in LI-treated patients
O'Malley Jr *et al.*[68] UP Phase II	Non-viral IL-2 gene immunotherapy Intra-lesional injection of cytokine genes	10 patients with unresectable or recurrent HNSCC	Confirmed safety of approach Phase II multicentre trial ongoing
Karcher *et al.*[42]* MCB Pilot study	Patients preconditioned with IL-2 & vaccinated with virus-modified autologous tumour cells Non-randomised trial	20 patients with stage III & IV HNSCC	Augmentation of DTH reactivity 5-yr survival rate 61% *vs* 38% in non-immunised group
Bonner *et al.*[63] NCI Phase III	Randomised study of radiotherapy ± concurrent cetuximab	Advanced SCC of oropharynx, hypopharynx or larynx 211 patients & 213 controls	Reduced mortality in treatment group No increase in common toxic effects associated with head & neck radiotherapy
Soulieres *et al.*[76] CHUM Phase II	Multicentre study of erlotinib, an oral epidermal growth factor receptor tyrosine kinase inhibitor	150 patients with local recurrent or metastatic HNSCC	Erlotinib well tolerated & produced prolonged disease stabilisation Rash & diarrhoea most common drug-related toxicities
Chang *et al.*[41]* DSO Pilot study	Generation of vaccine-primed lymphocytes for head & neck cancer treatment	6 patients with advanced head & neck cancer (this includes all tumours not just the squamous cell carcinomas)	Increased CD4 + ve & CD8 + ve responses
Borjesson *et al.*[74]* VU Phase I	Radioimmunotherapy with ^{186}Re-labelled chimeric monoclonal antibody U36	20 patients with advanced HNSCC	Well tolerated in all patients, without significant systemic signs of toxicity Dose stable disease in 3 patients at MTD of 50 mCi/m^2
Colnot *et al.*[75] AN Phase I	^{186}Re-labelled chimeric monoclonal antibody U36	13 patients with recurrent or metastatic HNSCC	Dose-limiting myelotoxicity at 41 mCi/m^2 1 patient showed stable disease for 6 mths 2 patients with dose-limiting myelotoxicity showed marked reduction in tumour size for short period

Data obtained by a comprehensive search of Pubmed, Ovid and Google search engines, the National Cancer Institute and the *cancerhelp.org.uk website, using the following key words: immunotherapy, clinical trials, head and neck, oral, hypopharyngeal, laryngeal, squamous cell carcinoma, and HNSCC. UMSM = University of Maryland School of Medicine, USA; MAGE = Melanoma antigen; HPV = human papilloma virus; HNSCC = head and neck squamous cell carcinoma; UMH = University of Michigan Hospitals, USA; IRX-2 = Iroquois homeobox 2 gene; NIO = National Institute of Oncology, Semmelweis University, Budapest, Hungary; LI = leukocyte interleukin; T = tumour; N = node; M = metastasis; CD = cluster of differentiation; +ve = positive; UP = University of Pennsylvania, USA; IL = interleukin; MCB = Molecular Cell Biology Group, University of Heidelberg, Germany; yr = year; DTH = Delayed-type hypersensitivity; NCI = National Cancer Institute USA & Comprehensive Cancer Centre University of Alabama, USA; CHUM = CHUM Hospital Notre Dame, Montreal, Quebec, Canada; DSO = Division of Surgical Oncology, 3302 Cancer Centre, Michigan, USA; VU = Department of Otolaryngology/Head and Neck Surgery, VU University Medical Center, 1081HV Amsterdam, The Netherlands; MTD = maximum tolerated dose; AN = University Hospital Vrije Universiteit, Amsterdam, The Netherlands; mths = months

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often skewed in favour of T helper-2 cell cytokines (see above). Briefly, this is due to a number of factors, including: poor generation of antibodies with a high affinity for tumour-expressed antigens; the fact that mammalian cells express a number of highly efficient anti-complement factors that largely prevent complement damage to host cells; and inefficient activation of the phagocytic effector cells by the cytokine repertoire. Although we have stated that the 'natural' B cell mediated immune response is relatively poor, it must be noted that the use of humanised monoclonal antibodies raised against key targets is now offering a realistic treatment options, e.g. Rituxan/MabThera® (anti-CD20 monoclonal antibody) in Non-Hodgkin's lymphoma and Herceptin® (anti-Human Epidermal Growth Factor (Her2)/neu monoclonal antibody) in breast cancer (F. Hoffmann-La Roche Ltd, Basel, Switzerland).[14,15] In head and neck SCC, much attention has focused on targeting epidermal growth factor receptor,[16] which is discussed below.

Tumour antigens

Although tumour cells are 'self cells', they express an altered repertoire of molecules, some of which are termed tumour-associated antigens. Eli *et al.*[17] have broadly classified these antigens as follows: normal cellular gene products expressed at an inappropriate time (e.g. oncofetal antigens and prostate-specific antigen); mutant cellular gene products (e.g. mutant p53 or RAS proteins); and viral gene products (e.g. E6 and E7 proteins of the human papilloma viruses 16 or 18).

A growing number of tumour-associated antigens have been identified in tumours such as prostate cancer and melanoma (e.g. melanoma-associated antigens); however, these molecules are not restricted solely to these tumours.[18] For instance, expression of both melanoma-associated antigens one and three has been reported in head and neck SCC; however, the clinical significance of this remains to be established.[19,20] Over the past few years, it has become clear that the process of antigen presentation is extremely important in initiating the immune response; this role is primarily performed by the dendritic cells.

Dendritic cells

Dendritic cells are naturally occurring antigen-presenting cells which specialise in initiating a primary immune response.[21] They exist in either immature form (mainly in non-lymphoid tissue) or mature form (in the T cell areas of lymphoid organs).[22] Mature dendritic cells have the ability to activate effectively both CD8 positive and CD4 positive T cells, which either mediate direct tumour cell cytotoxicity or alter the cytokine environment to promote cytotoxic T lymphocyte activity or anti-tumour antibody production by B cells.[23–25]

Defects in dendritic cell function have been reported at many different points along the pathway of cell development and antigen presentation. Firstly, Almand *et al.*[26] demonstrated defective differentiation of mature dendritic cells in head and neck SCC, which correlated with poor prognosis; they later showed that this was mediated via reduced T cell stimulation.[27] Interestingly, these effects were observed in patients with non-small cell lung carcinoma and breast cancer as well as in those with head and neck SCC.[27] Furthermore, Tas *et al.*[28] and Kerrebijn *et al.*[29] both demonstrated impairment of the chemotaxis and clustering ability of dendritic cells in patients with head and neck SCC. The cause (or causes) of dendritic cell malfunction is not clear, although there is good evidence to support a role for the tumour itself, with one of the likely soluble mediators being tumour-secreted interleukin 10. This key T helper-2 cell cytokine has been shown to have multiple inhibitory effects on dendritic cells, including: blocking differentiation from monocytes;[30] impairing dendritic cell maturation;[31] and inhibiting the primary allogeneic T cell response to human epidermal Langerhan's cells.[32]

Interleukin 10 is not the only cytokine involved in modulating dendritic cell function. A number of other tumour-secreted factors have been cited in this role, including vascular endothelial growth factor, granulocyte macrophage colony stimulating factor and low molecular mass factors.[33,34] Strauss *et al.*[34] showed that dendritic cells incubated with tumour supernatant from head and neck SCC or vascular endothelial growth factor A differentiated into immature dendritic cells and did not develop full stimulatory activity. Vascular endothelial growth factor, granulocyte macrophage colony stimulating factor and low molecular mass factor are multifunctional, enhancing angiogenesis, tumour progression, immunosuppression and immune tolerance; therefore, they are likely to act at many different levels to facilitate tumour development and/or progression.

In general, when mature dendritic cell levels are increased there is an improved clinical outcome, and when levels are low there is a poorer outcome. A higher number of dendritic cells infiltrating a tumour has been shown to be highly significant as a positive prognostic marker. This was well demonstrated by Goldman *et al.*, [35] who studied 43 patients with SCC of the tongue and showed that increased dendritic cell density in the peri-tumoural region correlated well with improved survival. Therefore, the aim of restoring or enhancing the recruitment of mature dendritic cells and of improving their function (either by direct stimulation and/or removal or inhibition of inhibitory factors) is very worthy of effort. However, to date only a few studies have been undertaken, with varying levels of success.[36,37]

All of the studies above have used advanced tumours. However, Nix and colleagues[38] have studied a large cohort of early laryngeal tumours, and have shown that there is no difference in the number of dendritic cells, comparing radioresistant and radiosensitive pre-treatment biopsies. Therefore, at least in the early stages of head and neck SCC, there is the potential benefit of developing a vaccination that can enhance the specific anti-tumour immune response.

Because of the enormous potential of dendritic cells, there is a growing interest in using them as

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the main part of a tumour vaccine, particularly in an attempt to treat disseminated micrometastasis. Unfortunately, despite the increase in our understanding of tumour immunology, no dendritic cell based therapy (or other immunologically based treatment) has yet entered the clinic. The current status of immunotherapy modalities is reviewed below.

Current immunotherapy treatment modalities in head and neck squamous cell carcinoma

Whiteside and colleagues[39] have attributed the difficulty in developing immune-based cancer therapies to two main factors: (1) active tumour escapes from the host immune system, and/or (2) failure of immune surveillance to control tumour progression.

Any form of immunologically based therapy must overcome these two obstacles. Thus, many research groups are seeking to involve multiple components of the immune system, in an attempt to recreate the body's original, integrated immune response. A number of key studies are highlighted below, in order to exemplify the approaches being actively investigated (see Table I).

T cell immunotherapy

Many early studies involved harvesting patients' T cells, activating and expanding these *in vitro*, and then infusing them back in an autologous manner. For example, To *et al.*[40] undertook a non-randomised, phase I clinical trial in 17 patients with advanced head and neck SCC (recurrent and metastatic disease) who had failed conventional treatment. In this study, patients were 'vaccinated' in the thigh with irradiated autologous tumour cells admixed with granulocyte macrophage colony stimulating factor. Eight to 10 days later, the draining inguinal lymph nodes were resected, and the resulting lymph node lymphocytes were polyclonally activated with the superantigen staphylococcal enterotoxin A and expanded in IL-2 *in vitro*. The resulting tumour-sensitised T lymphocytes, a mixture of CD4 positive and CD8 positive cells, were then infused back into the patients. Although the study cohort was small, the results were extremely encouraging, with one of the patients having no evidence of disease four years after surgical resection of a vertebral body metastasis and three others having their progressive disease stabilised. The toxic effects associated with immunisation were minimal and only affected four patients. This study demonstrated a safe procedure, and results from phase II trials are awaited with interest.

A more recent *in vivo* study by Chang *et al.*[41] showed that T cell responses could be induced by autologous tumour vaccination. In this study, six patients with recurrent head and neck SCC were injected intradermally with irradiated autologous tumour cells mixed with bacillus Calmette–Guerin. Although measurable increases were seen in the CD4 positive and CD8 positive responses, all of the patients had progressive disease, with only one patient showing an initial measurable decrease in the size of their recurrent neck mass. However, one key finding of this study was that the treatment appeared safe, and it has been hypothesised that this approach would be applicable for patients with earlier stage disease, whose immune system was less compromised.

Modified autologous tumour cell vaccine

In a recent, non-randomised, clinical trial, Karcher *et al.*[42] used a virus-modified, autologous tumour cell vaccine in 20 patients with advanced head and neck SCC (stage III and IV tumours). The virus was added to the vaccine in an attempt to stimulate the immune system more efficiently. In patients who were preconditioned with interleukin 2 (IL-2) and then vaccinated with virus-modified, autologous tumour cells, the investigators demonstrated an increased number of T cells and near-normal mitogenic stimulation capacity of these cells. Anti-tumour reactivity was determined by a delayed-type hypersensitivity skin reaction, a manifestation of a T cell mediated response commonly used to monitor immunotherapy studies. The five-year survival rate for patients receiving the vaccine was 61 per cent,[42,43] which was significantly better than the figure of 38 per cent reported by Gleich and colleagues for a cohort of 363 head and neck SCC patients of similar subgroup who received conventional treatment.[44]

Dendritic cell based vaccines

The outstanding ability of dendritic cells to initiate a primary immune response is the basis of current efforts to produce dendritic cell vaccines for patients with head and neck SCC. Put simply, the aim is to prime these cells with tumour-associated antigens and then subsequently to 'vaccinate' the patients, hoping to stimulate a strong, tumour-specific immune response that will cause regression, or at least tumour stasis.

A number of dendritic cell vaccines are currently being tested in colorectal, lung and renal cancers and in multiple myeloma, but there is little current clinical work on head and neck cancer. However, a variety of *in vitro* strategies have been proposed. Weise *et al.*[45] have demonstrated that a vaccine can be made by hybridising mature dendritic cells with a laryngeal carcinoma cell line (UTSCC-19A), although this needs to be tested to assess whether it will induce specific cytotoxic T lymphocytes *in vivo* in all patients. More recently, Kacani *et al.*[46] have reported that a vaccine comprising dendritic cells and necrotic cells from head and neck SCC cell lines is a suitable strategy for adjuvant immunotherapy in head and neck SCC. Their study demonstrated the induction and maturation of dendritic cells and subsequent production of IL-12 by this vaccine. The production of IL-12 is significant, as this pivotal T helper-1 cell cytokine will directly activate natural killer cells and promote T cell differentiation into cytotoxic T lymphocytes. We believe that dendritic cell based vaccines will play a key role in head and neck SCC treatment in the coming decade.

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p53 and p53 vaccines

The p53 gene is the most commonly mutated gene in all cancers, including head and neck SCC; this mutation subsequently leads to over-expression of a mutant form of the protein (p53). Normally, p53 protein exists in the cell at very low concentrations. The protein accumulates during times of cellular stress, leading to arrest of the cell cycle, allowing time for repair of the incurred damage or, if damage is irreparable, apoptosis of the cell.[47] This arrest occurs at the G1 and G2 phase of the cell cycle.[48] Therefore, mutation of the p53 gene results in the loss of this 'guardian of the genome' function.

Various studies[48–50] have shown that the introduction of wild-type p53 gene has significant effects, and there have been some promising results in terms of tumour therapy. There is evidence that human leukocyte antigen (HLA; the nomenclature for the human MHC molecules) A2-restricted cytotoxic T lymphocytes specific for human wild-type sequence p53 epitopes lyse tumour cells expressing mutant p53.[51–53]

It is on this basis that Hoffmann *et al.*,[54] using cytotoxic T lymphocytes generated *ex vivo* from circulating precursor T cells, evaluated their cytolytic ability in a cohort of 30 HLA-A2.1 positive head and neck SCC patients, together with 31 non-tumour controls. Patients were divided into two groups based on low or no p53, as compared with subjects with normal levels. The group of patients with low or no p53 effectively generated cytotoxic T lymphocytes specific for a wild-type p53 peptide (264–272 amino acids), whereas the subjects with normal levels did not. Flow cytometric analysis using HLA-A2.1 tetramers with this specific p53 peptide confirmed that the patients in the former group had relatively high percentages of CD3 positive CD8 positive cytotoxic T lymphocytes, in contrast to those patients with normal levels of p53. Hoffman suggested that p53-specific cytotoxic T lymphocytes could be generated *in vivo*, which could eliminate tumour cells expressing the relevant peptide epitope; however, this may allow the expansion of 'epitope-loss' tumour cells. The logical deduction from these results would be that a polyclonal response needs to be generated, i.e. induction of multiple cytotoxic T lymphocyte clones reacting with an array of epitopes; this is what the immune system does naturally when responding to foreign micro-organisms. It has also been suggested that more immunogenic variant peptides of the p53 peptide could be used to induce patients' cytotoxic T lymphocytes which were otherwise non-responsive; other studies have demonstrated similar outcomes and support these findings.[55,56] Because of the high prevalence of p53 mutations, immunisation strategies similar to those described above remain the subject of active research; however, transfer of results to the clinic will need time.

Deoxyribonucleic acid vaccines

Finally, it is important to highlight the attempts being made to produce deoxyribonucleic acid (DNA) vaccines, which aim to introduce genetic material into cells to induce expression of specific tumour-associated antigens, against which a patient's T and B cells can respond appropriately. The advantage of this approach is that DNA vaccines are relatively robust and simple to construct, and they harness the body's own protein production mechanisms. Therefore, as long as sufficient antigen can be produced, which is the most difficult challenge of using this approach, an active immune response should be efficiently generated.[57] Deoxyribonucleic acid vaccines have been shown to be remarkably good immunogens for inducing cellular immune responses, as they are able to activate all facets of the immune system, including cell-mediated killing, cytokine release and the production of antigen-specific antibodies.[58,59] The development of DNA vaccines for head and neck SCC is at an early stage. However, in tumours such as prostate cancer, malignant melanoma and human papilloma virus related tumours (such as cervical cancer), clinical trials of DNA vaccines are at an advanced stage.[60–62]

Monoclonal antibodies

Since 1975, when Kohler and Milstein first described the process of making monoclonal antibodies, there has been much hope that these 'magic bullets' would be able to specifically target and destroy cancers. During the past 30 years, there have been many false hopes. However, with the advent of molecular biology techniques that have facilitated the relatively simple production of humanised reagents, monoclonal antibodies are now finally realising their potential for the treatment of many tumours, e.g. the use of Herceptin in breast cancer and Avastin™ (F. Hoffmann-La Roche Ltd, Basel, Switzerland) targeting vascular endothelial growth factor. In the case of head and neck SCC, one of the most obvious target molecules is epidermal growth factor receptor, as this is over-expressed in the vast majority of head and neck tumours, even at early stages of development.[16] As its name suggests, epidermal growth factor receptor provides a growth signal on ligation; thus, blocking epidermal growth factor receptor with monoclonal antibodies has long been considered a logical course of action. Cetuximab (or Erbitux®; ImClone Systems Incorporated, Branchburg NJ, USA.) is a humanised monoclonal antibody that binds and blocks epidermal growth factor receptor signalling; early studies have suggested that treatment with this reagent boosts the effectiveness of radiation therapy in patients with head and neck SCC.[63]

Again following the concept that using a combination of therapeutic approaches is better than a single point of attack, clinical studies on the use of tyrosine kinase inhibitors are also ongoing. Tyrosine kinases are a group of enzymes involved in transducing signals from the cell surface receptors into the nucleus, where the appropriate response is made. Epidermal growth factor receptor, in common with many growth factor receptors, utilises tyrosine kinases which can be effectively blocked by drugs such as Iressa™ (F. Hoffmann-La Roche Ltd,

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Basel, Switzerland), which is currently undergoing phase III UK trials for advanced head and neck SCC.[64] There are an increasing number of 'small molecule' drugs (such as tyrosine kinase inhibitors) being developed, with great potential for applications in cancer therapy; because these drugs target the underlying cellular mechanisms, they may become even more important than antibodies in the future. The one major limitation of this group of molecules is how to introduce them selectively into the tumour cells; this is an area of active research by many groups.[65–67]

Cytokines

As is clear from Figure 2, cytokines play a key role in controlling and modulating all parts of the immune system. If the 'incorrect' cytokine environment is predominant, key cells are not able to function effectively; that is, in the presence of a T helper-2 cell cytokine milieu (i.e. raised concentrations of IL-4 and IL-10), dendritic cells cannot mature and/or present antigen efficiently, and cytotoxic T lymphocytes respond poorly against the tumour targets. One obvious response to this is to attempt to correct the cytokine imbalance by direct administration of the relevant, desired cytokines. However, a major drawback is once again the difficulty in targeted delivery, as the anti-tumour immune response needs to be localised rather than systemic. Intra- or peri-lesional injection of various cytokines has emerged recently as a promising technique for the treatment of head and neck SCC, but further confirmatory studies are required.[68]

A study by Van Herpen *et al.*[69] in a phase II trial involved intra-tumoural administration of recombinant IL-12 in 10 previously untreated patients with head and neck SCC (oral cavity or oropharyngeal tumours (staged as tumour $(T)_{1-4}$, node $(N)_{0-2}$ and metastasis $(M)_0$). Patients were given dose levels of 100 ng and 300 ng IL-12/kg, two or three times once weekly, before surgery. This group was compared with a control group of 20 patients (not treated with IL-12). Both groups underwent surgical

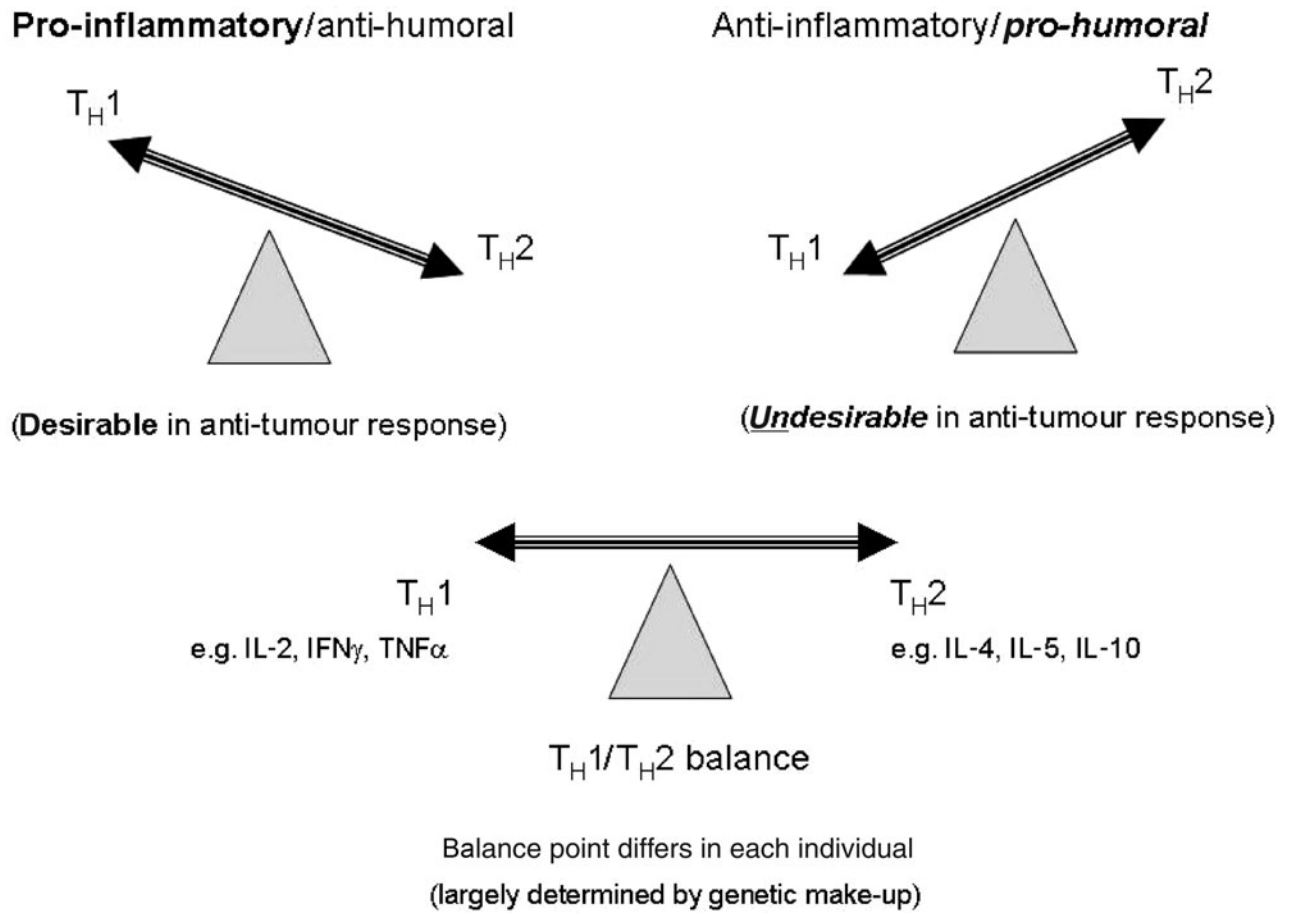

FIG. 2

T cell subsets and cytokine balance. T_H1 = T helper-1 cell; T_H2 = T helper-2 cell; IL = interleukin; IFN = interferon; TNF = tumour necrosis factor

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resection, including a supraomohyoid or radical neck lymph node dissection. When compared with the control group, the patients receiving IL-12 showed measurable, local regional immunological responses; however, there was toxicity, particularly apparent at the higher dose levels of 300 ng/kg, which limited the duration of the study. A dose-dependent increase in plasma interferon γ and IL-10 was also detected, together with a redistribution of lymphocytes from the peripheral blood to the enlarged lymph nodes in the neck, highlighting the impact on the wider immune system.

A further approach that has been investigated is to combine intra-lesional administration of cytokines with chemotherapy. This approach is best demonstrated by Timar *et al.*, [70] in a phase II, multicentre trial using a local, neoadjuvant leukocyte IL injection regimen in oral SCC (T_{2-3}, N_{0-2} and M_0), together with low dose cyclophosphamide, indomethacin, zinc and multivitamins. This study concluded that '[local neoadjuvant leukocyte IL injection] treated oral SCC patients were characterised by a markedly altered composition of tumour-infiltrating mononuclear cells, increased CD4/CD8 ratio, and increased tumour stroma to epithelial ratio, all of which were distinct from controls'. Promising results have been reported from several other similar studies and clinical trials using intra-lesional cytokine injections, especially where these injections have been used in combination with other modalities of head and neck SCC treatment.[68,71]

Conclusion

The multi-faceted immune system, working in a coordinated manner, is both highly efficient and highly effective at dealing with foreign invaders, e.g. bacteria and viruses. As we learn more about the individual contributions of different components, our attempts at harnessing this system against the altered self cells of tumours become more effective. It has taken approximately 30 years for antibodies to begin to show their therapeutic worth; thus, one must not be surprised that the early work on dendritic cells and T cell based strategies has not yet yielded reproducible clinical therapies. One factor that must never be forgotten when considering head and neck SCC is that it is a mixture of quite different diseases (e.g. oral SCC behaves very differently to laryngeal SCC), and evidence is emerging that the immune response against these tumours is different. Hence, it is unlikely that one immunotherapy will be effective for all head and neck SCC, and researchers must be careful to reflect this in their patient cohorts under study. We fully believe that the combination of immune components or immune factors with conventional therapy (chemo- or radiotherapy) will offer the most likely avenues for success in the not so distant future.

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