Anti-CD3/Anti-CD28 Bead Stimulation Overcomes CD3 Unresponsiveness in Patients With Head and Neck Squamous Cell Carcinoma

Terry Y. Shibuya, MD; Wei-Zen Wei, PhD; Michelle Zormeier, MD; John Ensley, MD; Wael Sakr, MD; Robert H. Mathog, MD; Robert J. Meleca, MD; George H. Yoo, MD; Carl H. June, MD; Bruce L. Levine, PhD; Lawrence G. Lum, MD

Objectives: To test whether T-cell CD3 responses are altered in patients with advanced-stage head and neck squamous cell carcinoma (HNSCC) and whether anti-CD3/anti-CD28 (αCD3/αCD28) bead stimulation could reverse CD3 unresponsiveness.

Design: Anti-CD3 (αCD3) monoclonal antibody immobilized on tissue culture plastic was used to stimulate lymph node mononuclear cells (LNMCs) and peripheral blood mononuclear cells (PBMCs) from patients with advanced-stage HNSCC. Proliferation, T-cell phenotype, and cytokines were measured during 8-day in vitro stimulation. Immune-enhancing properties of αCD3/αCD28 beads were also tested on LNMCs and PBMCs. Cytotoxicity of bead-activated T cells (ATCs) was measured against autologous and allogeneic HNSCC.

Results: Six patients were nonresponders to αCD3 stimulation defined by tritium (3H) incorporation of less than 3500 cpm, whereas 11 patients were responders with 3H incorporation of 3500 cpm or more. Responders produced higher levels of interleukin (IL)–12 and interferon γ (IFN-γ) after αCD3 stimulation than nonresponders. No phenotypic or clinical differences were identified between groups. Stimulation with αCD3/αCD28 beads enhanced IFN-γ and IL-2 produced by both groups. Bead ATCs were generated from PBMCs of patient 11 in the responder group and lysed (± SD) 100% ± 1% of autologous tumor and 49% ± 1% of allogeneic tumor. Bead ATCs from LNMCs of this patient lysed 58% ± 1% of autologous tumor and 63% ± 1% of allogeneic tumor.

Conclusions: A subpopulation of patients with HNSCC who are nonresponders to αCD3 stimulation has been identified, showing reduced proliferation and IL-12 and IFN-γ secretion. Nonresponders stimulated with αCD3/αCD28 beads reversed immune unresponsiveness and induced a type 1 cytokine response. Bead-generated ATCs from patient 11 in the responder group lysed autologous and allogeneic HNSCC in vitro, suggesting a possible effective immunotherapeutic modality in the treatment of HNSCC.


---

The induction of a T-cell immune response by antigen-presenting cells (APCs) occurs in 3 distinct stages. Initially, a nonspecific adhesion occurs between an APC and a T cell; next, the antigen–major histocompatibility complex of the APC crosslinks with the T-cell receptor (TcR). The final step occurs when a second or costimulatory signal is delivered by the APC to the T cell, enhancing stimulation. At present, the best-characterized second signal occurs when the B7.1 or B7.2 ligand of the APC binds to the CD28 receptor of the T cell, resulting in enhanced cellular activation. The immune response initiated may be characterized as a type 1 (T12) or 2 (T1c2) response based on the cytokines secreted from CD4+ T cells. A T11 response is characterized by the secretion of interleukin (IL)–2, tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ) and represents a delayed hypersensitivity type of response or cytotoxic response against cancer or microbes. A T12 response is characterized by the secretion of IL-4, IL-5, IL-6, IL-10, and IL-13 and enhances antibody production from B cells as well as an allergic response.

A hierarchy of immunosuppression exists in patients with head and neck squamous cell carcinoma (HNSCC). Immune reactivity is maximally suppressed in tumor-infiltrating lymphocytes, followed by proximal lymph node lymphocytes (LNL), distal LNL, and peripheral blood lymphocytes (PBL). The mechanism of immunosuppression has not been clearly defined. Recent studies from several laboratories suggest that altered expression and function of signal-transducing molecules associated with the TcR or CD3 are responsible for the immune deficiencies ob-
SUBJECTS AND METHODS

All individuals participating in our study provided informed consent, and a detailed explanation of the procedure, risks, and alternatives was given. The Human Investigation Committee and Institutional Review Board of Wayne State University, Detroit, Mich, granted approval for this study.

PATIENTS WITH HNSCC

Patients with advanced-stage HNSCC were studied because of the known immunosuppression that occurs with this cancer.1 Mean age of patients studied was 53.8 years, with a range of 38 to 68 years. All patients had advanced stage III (n = 5) or IV (n = 12) HNSCC. Primary sites of HNSCC included the oropharynx (n = 8), larynx (n = 5), oral cavity (n = 2), hypopharynx (n = 1), and an unknown primary site (n = 1).

PERIPHERAL BLOOD MONONUCLEAR CELLS

Peripheral blood was drawn from patients before surgery. Blood was suspended in an equal volume of phosphate-buffered saline solution, and peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation over a Ficoll-Hypaque density gradient (Pel-Freez, Brown Deer, Wis) for 10 minutes at 2400 rpm.

LYMPH NODE MONONUCLEAR CELLS

Lymph nodes were harvested at the time of surgery and placed in balanced salt solution with 20% heat-inactivated fetal calf serum, 1% penicillin (Gibco, Paisley, Scotland) and streptomycin (Gibco), glutamine (Gibco), penicillin (100 U/mL; Gibco), streptomycin (100 µg/mL; Gibco), and amphotericin B (100 U/mL; Gibco). For phenotyping and cytokine quantification, 7.5 x 10^5 PBMCs or LNMCs were cultured in 2.0 mL of culture media in 24-well flat-bottom plates for 6 days. All cells were cultured at 37°C and 5% carbon dioxide.

LYMPHOCYTE ACTIVATION

Lymphocytes were incubated in triplicate with uncoated plastic, CD3-coated plastic, and CD3/CD28–coated bead for 8 days. The PBMCs (1.5 x 10^6) were cultured in 200 µL of culture media in 96-well flat-bottom plates (Costar, Cambridge, Mass). Culture media consisted of RPMI 1640 (Gibco) supplemented with 10% fetal calf serum, 2-mmol/L glutamine (Gibco), penicillin (100 U/mL; Gibco), streptomycin (100 µg/mL; Gibco), and amphotericin B (100 U/mL; Gibco). For phenotyping and cytokine quantification, 7.5 x 10^5 PBMCs or LNMCs were cultured in 2.0 mL of culture media in 24-well flat-bottom plates for 6 days. All cells were cultured at 37°C and 5% carbon dioxide.

PLASTIC STIMULATION

The 96- and 24-well microtiter plates were precoated with αCD3 at a concentration of 2 µg/mL in phosphate-buffered saline solution for 4 to 18 hours at 37°C and washed served in several malignant neoplasms.4,8 To test whether altered CD3 or TcR responses exist in patients with HNSCC, we stimulated LNL and PBL from patients with advanced-stage HNSCC with anti-CD3 (αCD3) monoclonal antibody (MoAb) and measured immune responses.

Several immunotherapeutic trials use αCD3 MoAb to stimulate T cells. The capacity of αCD3 to stimulate T cells is enhanced when combined with anti-CD28 (αCD28 or 9.3) MoAb, which substitutes for the B7.1 or B7.2 ligand to stimulate selectively the CD28 receptor.9,12 A recent report13 has substantiated the importance of the B7.1 ligand and CD28 receptor in providing tolerance of the B7.1 ligand and CD28 receptor in providing B7.2 ligand to stimulate the CD28 receptor. Unlike the natural ligand, αCD28 MoAb specifically binds to the CD28 receptor in the absence of binding to cytotoxic T-lymphocyte antigen–4 (CD152), a negative regulator of the immune response.14 Anti-CD3/anti-CD28 (αCD3/αCD28) immobilized on iron beads has been used to overcome immunosuppression and generate bead-activated T cells (ATCs).15,16 Phase 1 adoptive immunotherapy trials using ATCs are being conducted in patients with human immunodeficiency virus (HIV), melanoma, lymphoma, and various solid tumors.17-19 In our study, αCD3/αCD28–coated beads were used to activate LNL and PBL from patients with advanced-stage HNSCC, and reversal of suppressed CD3 responses was measured.

RESULTS

T-CELL RESPONSES TO PLASTIC-ADHERENT αCD3

Anti-CD3 MoAb was immobilized on tissue culture plastic and used to stimulate LNMCs and PBMCs in vitro from patients with HNSCC. We chose CD3 because altered signaling via the TcR has been reported in other cancers. The optimal CD3-stimulating conditions were determined previously by proliferative responses of healthy control PBMCs.20 The LNMCs from 17 patients with advanced-stage cancer were stimulated with αCD3-coated plastic during an 8-day period. Eleven patients responded to αCD3 with [3H]-thymidine incorporation of 3500 cpm or more within the first 6 day of stimulation. They were defined as responders. Six patients did not respond to αCD3 and had [3H]-thymidine incorporation of less than 3500 cpm; they were defined as nonresponders (Figure 1). The difference in LNMC response to αCD3 stimulation between responders and non-
BEAD STIMULATION

The αCD3/αCD28–coated bead stimulation was performed at a 3:1 bead-lymphocyte ratio. For all samples, unstimulated controls were plated. Previous studies have shown that αCD28 alone and uncoated beads do not stimulate lymphocytes.20

[3H] THYMIDINE INCORPORATION BY PBMCs

On days 2, 4, 6, and 8, the cultures were pulsed with 7.4 × 10^4 Bq of [3H] thymidine (tritium thymidine) for 4 hours and harvested onto glass fiber discs using a cell harvester (PBD; Cambridge Technology, Cambridge, Mass). The glass fiber discs were counted in vials containing 6 ml of scintillation-counting cocktail in a scintillation counter (Beckman, Fullerton, Calif).

PHENOTYPIC ANALYSIS

On days 0 and 6 after incubation, cell suspensions were prepared from LNMCS or PBMC cultures and stained with MoAb to CD3, CD4, CD8, CD28, or CD45RO conjugated to phycoerythrin (PharMingen, San Diego, Calif) as previously described.21 Surface marker expression was measured by flow cytometry (FACScan; Becton Dickinson, San Jose, Calif).

CYTOKINE ANALYSIS

Cell culture supernatants were harvested on days 2, 4, 6, and 8. The quantity of IL-2, IL-4, TNF-α, IL-12, and IFN-γ present in the supernatant was determined using an enzyme-linked immunosorbent assay (ELISA) (R & D Systems, Minneapolis, Minn).

CYTOTOXICITY

Cytotoxicity was measured using a chromium 51 (51Cr) release assay. Activation of LNMCS or PBMCs was performed using αCD3/αCD28–coated beads for 6 days. Autologous and allogeneic HNSCC lines were labeled with 51Cr for 1 hour at 37°C with 3.7 × 10^4 Bq per 1 × 10^6 cells and washed 3 times. Varying numbers of effectors were then incubated with targets in a 96-well round-bottom plate in triplicate. All experiments were performed in triplicate. Supernatants from stimulated LNMCS or PBMCs were also tested against targets. Spontaneous release was determined by adding media only. Total releasable count was determined by adding 0.2 N hydrochloric acid. Incubations were for 4 and 18 hours at 37°C. Culture supernatant was harvested using a harvesting system (Skatron, Lier, Norway).

Cytolytic activity (percent of specific 51Cr release) was calculated as follows:

\[
\text{Percentage of Specific } 51\text{Cr Release} = \left(\frac{\text{Exp.} - \text{Spt.}}{\text{Tot.} - \text{Spt.}}\right) \times 100
\]

where Exp, Spt, and Tot indicate experimental, spontaneous, and total releasable 51Cr, respectively.

STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) and t test were used to compare between groups, using the statistical package from Sigma Stats (Jandel Scientific, San Raphael, Calif).

CHARACTERIZATION OF RESPONDERS AND NONRESPONDERS

To identify differences between both groups in T-cell subpopulations, lymph node and peripheral blood T cells were phenotyped before αCD3 stimulation. The percentage of CD3, CD4, CD8, CD28, and CD45RO T cells was comparable in responders and nonresponders (Table 1). Shown in Table 1 is the phenotype of cells stimulated with αCD3/αCD28 beads. Stimulation with αCD3 alone did not allow for adequate expansion of T cells for phenotypic analysis.

Cytokine expression from responders and nonresponders was also significantly different (t test, \(P = .008\) for day 4 and \(P = .001\) for day 4 and \(P = .002\) for day 6) (Figure 1). Comparison of PBMC stimulation between responders and nonresponders was significant (t test, \(P = .03\) for day 4 and \(P = .001\) for day 4 and \(P = .008\) for day 6) (Figure 2).

αCD3/αCD28 BEAD COSTIMULATION OF NONRESPONDERS AND RESPONDERS

Because of the poor proliferation and IFN-γ production in nonresponders, we performed αCD3/αCD28 bead stimulation to test whether immune unresponsiveness could be reversed. Costimulation with αCD3/αCD28 beads is presently used in several adoptive immunotherapeutic trials to enhance immune function against infection and cancer. The responders functioned as a comparison group for nonresponders. Proliferative responses were measured over an 8-day period during bead stimulations. Significantly higher proliferation continued to occur in LNMCS on day 4 in responders compared with nonresponders (\(P = .02\), t test) (Figure 3). Differences between responders and nonresponders on day 6 were
reduced to insignificant levels (Figure 3). Differences between responders and nonresponders in PBMC proliferation on day 4 and 6 were reduced to insignificant levels (Figure 4). Proliferative deficiency of nonresponders to αCD3 stimulation was partially reversed by αCD3/αCD28 bead stimulation.

To identify differences in T-cell subpopulations between groups, lymph node T cells were phenotyped after αCD3/αCD28 bead stimulation. Expression of T-cell CD3, CD4, CD8, CD28, and CD45RO from responders and nonresponders was measured and compared on day 6. Similarly, peripheral blood T cells were phenotyped after αCD3/αCD28 bead stimulation and compared on day 6. There was a significantly higher percentage of CD3+ cells in nonresponder lymph node and peripheral blood T cells after αCD3/αCD28 stimulation compared with responders (Table 1). No other differences were identified.

To compare the immunologic environment existing in responders and nonresponders after αCD3/αCD28 bead stimulation, LNMCs and PBMCs were stimulated in vitro, and the production of IL-2, IL-4, IL-12, IFN-γ, and TNF-α was measured on day 6 (Table 2). There were no significant differences in cytokines produced by responders and nonresponders. Bead stimulation enhanced IL-2, IFN-γ, and TNF-α secretion in both responder lymph node and peripheral blood T cells after αCD3/αCD28 stimulation was significantly higher in responders (P = .002 for day 6). 

αCD3/αCD28 BEAD VS αCD3-COATED PLASTIC STIMULATION

Stimulation with αCD3/αCD28 beads was compared with αCD3 plastic stimulation for efficacy in inducing a Th1 immune response characterized by high levels of IL-2 and IFN-γ secretion. A Th1 immune response is highly desirable in fighting cancer. Bead stimulation significantly enhanced levels of IL-2 and IFN-γ in responders (IL-2, P = .001, t test; IFN-γ, P = .005) and IL-2 in nonresponders (P = .05) compared with αCD3 plastic stimulation (Table 2). Stimulation of αCD3/αCD28 beads was more effective than that of αCD3-coated plastic in producing TNF-α in responders (P = .005, t test) (Table 2).

CYTOTOXICITY OF αCD3/αCD28 ATCs

To further investigate the immune-enhancing properties of αCD3/αCD28 bead stimulation, the cytotoxicity of bead-generated ATCs was measured against autologous tumor. Patient 11 from the responders was chosen because tumor had been successfully cultured, and sufficient numbers of LNMCs and PBMCs were available for study. The ATCs were generated from responder 11 by stimulating LNMCs and PBMCs for 6 days in vitro with coated beads. Tumor cell lysis was measured using an 18-hour 51Cr release assay with effector-target ratios of 50:1, 25:1, and 12:1, respectively. Lymph node ATCs showed lysis (±SD) of 55% ± 1%, 58% ± 1%, and 44% ± 1%, respectively (Figure 5). Peripheral blood ATCs showed lysis (±SD) of 54% ± 1%, 58% ± 1%, and 48% ± 1%, respectively.
showed lysis (±SD) of 100% ± 1%, 100% ± 1%, and 89% ± 1%, respectively (Figure 5). Unstimulated lymph node and peripheral blood T cells showed minimal lysis (range, 0%-4%) (Figure 5). A 4-hour 51Cr release assay was also performed, which showed minimal lysis (range, 0%-2%). Supernatant from bead-activated peripheral blood and lymph node ATCs was tested against autologous tumor and showed minimal lysis (range, 0%-10%). Lysis of allogeneic tumor showed that killing was partially nonspecific in nature.

### Table 2. Cytokines Secreted on Day 6 by Responders and Nonresponders*

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>LNMC, pg/mL</th>
<th>PBMC, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Responders</td>
<td>Nonresponders</td>
</tr>
<tr>
<td>IL-2</td>
<td>0 ± 0</td>
<td>2 ± 4</td>
</tr>
<tr>
<td>IL-4</td>
<td>6 ± 36</td>
<td>81 ± 112</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>60 ± 167</td>
<td>58 ± 138</td>
</tr>
<tr>
<td>IL-12</td>
<td>12 ± 19</td>
<td>1 ± 3</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0 ± 0</td>
<td>50 ± 113</td>
</tr>
<tr>
<td>After αCD3 Plastic Stimulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>6 ± 7†</td>
<td>17 ± 16</td>
</tr>
<tr>
<td>IL-4</td>
<td>58 ± 113#</td>
<td>52 ± 94</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>266 ± 254‡</td>
<td>138 ± 188</td>
</tr>
<tr>
<td>IL-12</td>
<td>179 ± 140‡</td>
<td>14 ± 17</td>
</tr>
<tr>
<td>TNF-α</td>
<td>31 ± 86‡</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>After αCD3/αCD28 Bead Stimulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>2153 ± 1309**</td>
<td>1481 ± 1215**</td>
</tr>
<tr>
<td>IL-4</td>
<td>21 ± 37</td>
<td>37 ± 46</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>1677 ± 851**</td>
<td>1074 ± 872**</td>
</tr>
<tr>
<td>IL-12</td>
<td>35 ± 39</td>
<td>23 ± 19</td>
</tr>
<tr>
<td>TNF-α</td>
<td>293 ± 298**</td>
<td>150 ± 248</td>
</tr>
</tbody>
</table>

*LNMC indicates lymph node mononuclear cells; PBMC, peripheral blood mononuclear cells; IL, interleukin; INF-γ, interferon-γ; TNF-α, tumor necrosis factor α; αCD3, anti-CD3 monoclonal antibody; and αCD28, anti-CD28 monoclonal antibody. Data are given as mean ± SD. Unless otherwise specified, n = 10 for responders; n = 6 for nonresponders.

†P < .05, t test, responders vs nonresponders.

‡N = 8.

§N = 3.

∥N = 4.

¶N = 2.

#N = 9.

**P < .05, t test, αCD3/αCD28 bead-stimulated vs αCD3-stimulated group.
Recent studies from several laboratories suggest that alterations in function and expression of signal-transducing molecules associated with CD3 are responsible for the immunodeficiencies observed in malignant melanoma and renal cell, ovarian, and colorectal carcinoma. To test whether altered CD3 or TcR responses exist in patients with HNSCC, we stimulated LNLs and PBLs from patients with advanced-stage cancer with αCD3 MoAb and measured immune responses. By this method, 2 populations of patients, responders and nonresponders based on LNL (Figure 1) and PBL responses (Figure 2), were identified. The presence of CD3 on the surface of LNLs and PBLs was checked and confirmed by flow cytometry (Table 1). Flow cytometric analysis revealed no differences in T-cell subpopulations between groups (Table 2), and there was no clinical difference between groups. Responders included 3 patients with stage III and 8 patients with stage IV HNSCC. Nonresponders included 2 patients with stage III and 4 patients with stage IV disease. There was no difference in tumor burden between groups. Further study will be required to identify the specific defect in CD3 response observed herein. Others have attributed altered CD3 responses to loss of CD3-ζ chain expression.

The characterization of responders revealed a difference in the spontaneous release of cytokines compared with nonresponders. A higher level of IL-12 was produced by PBMCs in responders compared with nonresponders (Table 2). Elevated IL-12 levels suggested a baseline Th1 immune environment existed in the responders (Table 2). It may be possible that APCs or B cells in responders secret higher levels of IL-12, predisposing this subset of patients to a Th1 response. There were no treatment difference between responders and nonresponders; all had advanced-stage HNSCC and had not previously received chemotherapy or radiation therapy. The LNLs and PBMCs cultured in these experiments were a mixture of T and B cells. Flow cytometry before culturing showed no significant difference in T-cell populations between groups (Table 1). Higher baseline levels of IL-12 in the immune environment correspond to enhanced production of IFN-γ during immune stimulation. No differences were identified in baseline levels of IL-4, a Th2 type of cytokine; expression of IL-10 and tumor growth factor β was not checked.

Stimulation of both groups with αCD3 revealed that responders produced higher levels of IL-12 and IFN-γ compared with nonresponders (Table 2). This demonstrated that the immune environment of responders favors a Th1 response, whereas nonresponders showed minimal response. Reduced response to αCD3 stimulation may be caused by alterations in the CD3-ζ chain function or the presence of immunosuppressive factors such as prostaglandin E2 or tumor growth factor β. The exact cause of immune unresponsiveness has not been identified.

To test whether αCD3/αCD28 bead stimulation can overcome the suppressed responses identified in nonresponders, we stimulated both groups and compared results. Stimulation with αCD3/αCD28 beads was chosen because this method of T-cell stimulation is being studied in adoptive immunotherapeutic trials for the treatment of HIV, melanoma, lymphoma, and various solid tumors. We have reported previously on the immunoenhancing properties of αCD3/αCD28–coated surgical suture. Stimulation with αCD3/αCD28 beads enhanced proliferation in both groups (Figures 3 and 4). Bead stimulation enhanced the production of IL-2, IFN-γ, and TNF-α in both groups (Table 2). Differences between responders and nonresponders in secretion of cytokines—spontaneously or after αCD3 stimulation—were reversed with αCD3/αCD28 bead stimulation. Bead stimulation generated more Th1 cytokine production in both groups (Table 2).

The cytotoxicity of bead-stimulated ATCs against autologous cancer was tested. Peripheral blood ATCs from responder 11 showed 100% ± 1% lysis of tumor at 50:1 and 25:1 effector-target ratios, with 89% ± 1% lysis at 12:1. Lymph node ATCs killed in the range of 50% to 60% (± 1%), with effector-target ratios of 50:1, 25:1, and 12:1, respectively. The specificity of kill was measured by using allogeneic HNSCC as targets. Peripheral blood and lymph node ATCs showed lysis of 23% to 49% (± 1%) and 22% to 63% (± 1%), respectively. Therefore, a por-
tion of tumor kill was nonspecific in nature. To test whether lysis resulted from a soluable factor, supernatant was tested against autologous and allogeneic tumor. Minimal lysis (0%-10%) was noted, indicating the absence of a soluable cytolytic factor. Cytotoxicity assays were performed for 4 and 18 hours. Four-hour incubations showed minimal lysis (0%-4%), whereas 18-hour incubations increased kill up to 100%. The significance of prolonging incubations is not clear. Cytotoxicity experiments were performed in only 1 patient (responder 11), because the availability of autologous tumor, LNMCs, and PBMCs was limiting. Cells from other individuals may have a different response.

The generation of a T₄₁ immune response makes this method of T-cell stimulation an attractive immunotherapeutic option for treating HNSCC. Cytokines showing T₄₁ have been associated with cytotoxic immune responses against cancer. In fact, our findings are consistent with those of other investigators who have found enhanced immune function with αCD3/αCD28 bead stimulation of cells from patients seropositive for HIV. In a phase 1 trial involving autologous reinfusion of CD₄+ T cells to patients who are seropositive for HIV, a long-term rise in CD4 counts has been observed (C.H.J., personal communication, May 1999). The in vitro human data presented in our study strongly support the development of a phase 1 adoptive immunotherapy trial to test αCD3/αCD28 bead-generated ATCs against advanced-stage HNSCC.

CONCLUSIONS

Subpopulations of patients with HNSCC have been identified who are nonresponders to αCD3 stimulation. Nonresponders showed reduced expression of IL-12 and IFN-γ compared with responders. Stimulation with αCD3/αCD28 beads overcame the CD3 unresponsiveness identified in nonresponders and generated a T₄₁ immune response. The αCD3/αCD28 bead-generated ATCs from responder 11 were able to lyse autologous and allogeneic HNSCC in vitro. Costimulation with αCD3/αCD28 beads generated ATCs that may be an effective immunotherapeutic modality in the treatment of HNSCC.

Accepted for publication November 16, 1999.

This study was supported by grant T32DC00026 from the National Institutes of Health, Bethesda, Md, and grant IN-162 from the American Cancer Society, Atlanta, Ga.

Reprints: Terry Y. Shibuya, MD, Department of Otolaryngology–Head and Neck Surgery, Wayne State University School of Medicine, 540 E Canfield, 5E-UHC, Detroit, MI 48201 (e-mail: TShibuya@med.wayne.edu).

REFERENCES


