CHARACTERIZATION OF CELLS FROM INVADED LYMPH NODES IN PATIENTS WITH SOLID TUMORS

Lymphokine Requirement for Tumor-specific Lymphoproliferative Response

BY FEDERICO COZZOLINO,* MARIA TORCIA,* ANNA MARIA CAROSSINO,* ROSANNA GIORDANI,* CESARE SELL,* GUGLIELMO TALINI,* EMANUELE REALI,† ANDREA NOVELLI,* VITO PISTOIA,* AND MANLIO FERRARINI

From the *Istituto di Clinica Medica 1, †Istituto di Clinica Urologica, ‡Istituto di Clinica Otorinolaringoiatrica, †Dipartimento di Farmacologia, Università degli Studi di Firenze; and the *Servizio di Immunologia Clinica, Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy

The cellular responses to tumor cells are mediated by a variety of cell types, such as T cells, NK or lymphokine-activated killer (LAK) cells, and macrophages (1–7). T cells possess the distinctive features of being clonally distributed and of recognizing the antigen on the target cell membrane in association with molecules coded for by genes of the MHC. Three major T lymphocyte subsets (helper, cytotoxic, and suppressor) participate in the response to malignant cells (1–3). The first two cell types may exert a function that is beneficial to the host, whereas suppressor T cells may facilitate tumor growth. The different T cell subsets can be recognized through the expression of distinctive surface markers and can also be tested in a variety of experimental systems. Nevertheless, there has been so far considerable difficulty in elucidating the types of cell interactions that occur during the response to cancer cells. Lack of knowledge in the field has impeded clarification of the mechanisms that lead to a failure of tumor rejection. This has been particularly true for the studies on human tumors, where only in vitro methods could be used. In addition, the prevalent use of peripheral blood as a potential source of tumor-specific T lymphocytes may have contributed to the difficulties encountered in these studies (as discussed in 8–10). In the peripheral blood, T cells with specificity for tumor antigens may be far too diluted, or else their activity may be masked by that of possibly more powerful cytotoxic effector cells such as LAK or NK cells.

In the present investigation, we have explored the possibility that lymph nodes draining the tumor site from patients with cancer of the larynx or of the bladder...
could contain tumor-specific T lymphocytes. These experiments were in part suggested by previous observations that these lymph nodes comprise T cells with particular and somewhat unusual surface phenotype (11).

We show that invaded lymph nodes contain T lymphocytes that can be induced to proliferate specifically in response to the patients' malignant cells. A positive response requires, however, that two basic conditions are met, namely that the cultures are supplemented with IL-1 and that a subset of suppressor T cells with a distinctive surface phenotype is removed from the suspensions.

Materials and Methods

_Lymph Nodes and Neoplastic Tissues._ Pelvic lymph nodes from 18 patients with transitional cell carcinoma of the bladder and cervical lymph nodes from 12 patients with spinal-cellular carcinoma of the larynx were obtained after informed consent. Each lymph node was divided into two parts for histopathologic evaluation and for in vitro studies. Fragments of tumor tissues were obtained from individual patients under sterile conditions.

_Antibodies and Surface Marker Analysis._ Anti-T cell mAbs OKT3, OKT4, OKT8 (12) were obtained from Ortho Diagnostic Systems, Milano, Italy. The OKM1 mAb, that recognizes the $\alpha$ chain of the C3bi receptor on monocytes, granulocytes, T cells, and NK cells (13), was also purchased from Ortho Diagnostic Systems. The monocyte-specific Leu-M3 mAb (14) and Leu-11b mAb, which detects the receptor for the Fc portion of IgG on granulocytes and NK cells (15), were purchased from Becton Dickinson Laboratory Systems, Milano, Italy. The B cell-specific Bl mAb (16) was obtained from Coulter-Kontron, Milano, Italy. HNK-1 clone, originally raised by Abo and Balch (17, 18), which produces the Leu-7 mAb, was obtained from the American Type Culture Collection, Rockville, MD. The mAb, which detects subpopulations of NK cells and of T cells, was purified from ascites fluid by filtration on a Ultrogel AcA 34 column. An mAb that reacts with the IL-2 receptor (anti-Tac) (19) was kindly provided by Dr. T. A. Waldmann, National Institutes of Health, Bethesda, MD. PTF 29/12 mAb, an anti-HLA-DR nonpolymorphic determinant (20), and 22.1 mAb, an anti-HLA-A,B,C monomorphic antigen (21), were generous gifts of Dr. G. Damiani, University of Genova.

F(ab$'$_2$)$ fragments of affinity-purified goat anti-mouse Ig (GAMIg) and their FITC-conjugated forms were prepared as previously described (22), and used for cell separation and indirect immunofluorescence analysis, respectively. The polyclonal rabbit anti-human IL-1 antiserum was purchased from Genzyme, Boston, MA.

Surface marker analysis of lymph node cells, or of the cell lines thereof, was carried out by conventional fluorescence microscopy, as described (22). For the quantitative evaluation of Tac antigen expression by cultured lymph node cells, a cytofluorographic analysis was carried out with a FACS (Becton Dickinson Laboratory Systems). 10$^6$ cells were treated at 4°C for 30 min with anti-Tac mAb (ascites, 1:1,000 final dilution), washed in HBSS, and incubated with F(ab$'$_2$)$ fragments of GAMIg for additional 30 min. An aliquot of freshly isolated cells, stained as above, was fixed in a 1% formaldehyde solution in HBSS, FACS analyzed after 3 d, and compared with cultured cells. Controls included cells treated with an unrelated monoclonal Ig of the same isotype. The data were expressed as a histogram in which the fluorescence intensity (log) was plotted on the abscissa against the relative number of cells on the ordinate. In parallel, volume-distribution histograms, based on forward light scatter, were plotted for each sample.

_Cell Separation Procedures._ Lymph node fragments were gently dissociated to obtain single-cell suspensions. Mononuclear cells were separated on Ficoll-Hypaque density gradients.

Cells that expressed the Leu-7 marker were fractionated by a direct rosetting technique with ox red cells coated with the purified Leu-7 mAb, as described (23). Cells bearing the OKM1 determinant were separated by indirect rosettes, according to Egeland and Lea (24). Briefly, 2 x 10$^7$ cells were resuspended in 400 $\mu$l of a 1:10 dilution of the OKM1 mAb in HBSS and kept at 4°C for 30 min. After two washes with cold HBSS, cells were
incubated with a 1% suspension of ox red cells coated with F(ab')2 fragments of GAM Ig at 37°C for 1 h. Rosetting cells were then separated by Ficoll-Hypaque density gradients, as described (23). Red cells were lysed by incubating at 4°C for 10 min with a 0.83% NH4Cl solution.

Peripheral blood mononuclear cells were fractionated into T cells and non-T cells by rosetting with neuraminidase (Sigma Chemical Co., St. Louis, MO)-treated sheep red blood cells, as described (23). Non-T cells were irradiated (3,000 rad) and used as stimulator cells against autologous T cells in a mixed lymphocyte reaction. In all of the above experiments, the rosette-enriched fractions contained ≥94% rosetting cells.

To isolate tumor cells, fragments of ~1 cm³ were cut from the tumor mass and mechanically dissociated in the presence of 0.2 mg/ml deoxyribonuclease (Sigma Chemical Co.). To remove large cell aggregates, the suspensions were purified on Ficoll-Hypaque gradients. Tumor cells were separated from tumor-infiltrating lymphocytes by Percoll density gradients, as described by Kedar et al. (25). Briefly, 3 × 10⁷ cells were layered onto a discontinuous gradient of 25, 15, and 10% Percoll in HBSS and centrifuged for 7 min at 25 g. The pellet, enriched in tumor cells, was recovered and washed twice; cytocentrifuge smears were prepared to assess the purity of the suspension, which always exceeded 95%. Aliquots of tumor cells were frozen in 90% FCS/10% DMSO (Sigma Chemical Co.) over liquid nitrogen.

**Cell Cultures.** To test the response of T lymphocytes to tumor cells, various numbers of isolated lymph node cells or fractions thereof were cocultured with irradiated (5,000 rad) autologous or allogeneic tumor cell suspensions in flat-bottom microtiter plates in a total volume of 0.2 ml of RPMI 1640 (Flow Laboratories, Milano, Italy) at 37°C in a humidified atmosphere with 5% CO₂. RPMI 1640 was supplemented with 10% FCS (Gibco, Mascia Brunelli, Milano, Italy), 2 mM L-glutamine (Sigma Chemical Co.), 100 µg/ml streptomycin, 100 IU/ml penicillin (RPMI-FCS). Unless otherwise stated, the cells were cultured for 6 d.

For the autologous mixed lymphocyte reactions, lymph node or peripheral blood cells (or fractions thereof) were cultured with irradiated (3,000 rad) peripheral blood non-T cells, as stimulator cells. 2.5 × 10⁶ responder cells were cultured with an equal number of stimulator cells, using the same conditions as above. Cultures were pulsed with 0.5 µCi of [³H]Thiouracil (sp act 25 Ci/mmol; Amersham, Prodotti Gianni, Milano, Italy), harvested 18 h later, and the radioactivity was determined in a liquid scintillation counter (Beckman Analytical, Milano, Italy).

Statistical evaluation of data was performed by Student’s t test.

Lymph node cells were stimulated with 1 µg/ml PHA (PHA-P; Wellcome, Pomezia, Italy) or cultured with rIL-2 (25 U/ml), kindly donated by Biogen S.A., Geneva, Switzerland, with purified IL-1 (Genzyme), or human rIL-1-β, a generous gift of Biogen S.A. (0.5-2 U/ml). These experiments were generally carried out by culturing 2.5 × 10⁴ cells/well in 96-well plates. Cell proliferation was assessed as above. When larger cell quantities were needed, the cultures were set up in 24-well plates, at 10⁶ cells/ml, in the same conditions as above, or, when indicated, in the presence of rIL-2 or of purified IL-1.

To obtain continuous T cell lines, 2.5 × 10⁵ OKM1<sup>+</sup>, Leu-7<sup>-</sup> cells from metastatic lymph node cells were cocultured in 24-well plates with 2 × 10⁵ irradiated autologous tumor cells in 1 ml of RPMI-FCS, containing 2 U of purified IL-1. After 6 d, the supernatant was removed and replaced with fresh medium containing 25 U/ml rIL-2; at this time, additional tumor cells were added. This procedure was repeated every third day.

To test the proliferative activity of the cell line, 2.5 × 10⁴ cells were cultured for 3 d in the above conditions, with autologous or allogeneic irradiated tumor cells and/or rIL-2. When indicated, the cultures were set up in the presence of PTF 29/12 anti-HLA-DR antibody or 22.1 anti-HLA-A,B,C antibody, 1:1,000 final dilution of ascites fluids.

**IL-2 Assay.** 4 × 10⁵ murine IL-2-dependent CTLL-2 cells in 100 µl of RPMI-FCS containing 2.5 × 10⁻⁴ M 2-ME were incubated with 100 µl of various dilutions of test supernatants, in triplicate, at 37°C. After 24 h, 0.5 µCi of [³H]Thiouracil were added, and incubation was continued for another 4 h. The incorporated radioactivity was assayed as
described above. The IL-2 U/ml were defined as the reciprocal of the supernatant dilution that induced 50% of maximal proliferation of CTLL-2 cells.

**IL-1 Assay.** IL-1 activity was tested by the thymocyte costimulation assay, as described (23). Briefly, thymuses were surgically removed from C3H/HeJ mice; single-cell suspensions were obtained and thymocytes (1.5 × 10^6 cells/well) were cultured in triplicate for 72 h in 96-well, flat-bottomed plates, in the presence of 1 μg/ml PHA alone, or PHA plus serial dilutions of the supernatants to be tested. Subsequently, the cells were pulsed with 1 μCi of [3H]TdR for 8 h, harvested, and counted. 1 U of IL-1 was defined as the amount of supernatant capable of doubling the [3H]TdR incorporation by 1.5 × 10^6 stimulated thymocytes.

**Cytotoxicity Assay.** Cytotoxic activity was measured in a conventional 4-h assay against 51Cr-labeled cells, as described (23). Briefly, 2 × 10^6 viable (>80%) tumor cells, or K562 cells, were resuspended in 0.5 ml of RPMI-FCS and incubated with 300 μl of Na_2CrO_4 (sp act 200–500 Ci/mmol; NEN, Du Pont Italia, Firenze, Italy) at 37°C for 60 min. The cells were washed three times, kept for an additional 60 min at 37°C, and subsequently washed twice. E/T ratios of 30:1, 10:1, 1:1 were selected. E/T mixtures were incubated for 4 h, were centrifuged, the supernatants were recovered, and their radioactivity was determined. Maximal 51Cr release was determined by lysing the labeled cells with 0.1 N HCl. The percent specific 51Cr release was calculated as: 100 × [(release in presence of effector cells) − (release from labeled cells)] / [(release in presence of HCl) − (release from labeled cells)]

Cold-target inhibition studies were performed by first incubating the effector cells with unlabeled target cells (effector/cold target ratio of 20:1) for 1 h at 37°C, then with 51Cr-labeled cells. The assay was then performed as above.

**Results**

**Response of Lymph Node Cells to Autologous Malignant Cells.** In an initial series of experiments, lymphocytes from lymph nodes with and without metastases were tested for their ability to proliferate in response to autologous malignant cells obtained from the primary tumor lesion (mixed lymphocyte-tumor culture [MLTC]). Lymph nodes were subdivided as having or not having metastases according to conventional histopathology. In these experiments, a significant proliferative response was never observed, although a number of variables, such as cell concentration, stimulator/responder cell ratio, duration of coculture period, were tried. Lymph node cells were also studied for their cytotoxic activity against 51Cr-labeled autologous neoplastic cells. Again, a cytotoxic response was never obtained (data not shown).

In principle, the failure to observe a response could be related to any of the following, albeit not mutually exclusive, conditions: (a) absence of a tumor antigen(s)-specific lymphocyte population; (b) requirement for exogenous lymphokines; (c) influence of suppressor mechanisms. These possibilities were investigated as follows.

Cells from both invaded and noninvaded nodes were cultured in the presence of exogenous IL-2, with or without autologous tumor cells. The cells gave a significant response to IL-2, which was not modified by the presence of malignant cells (Table I). The response induced by exogenous IL-2 was not unexpected, because a number of cells stained strongly for Tac antigen (see below and Cozzolino et al. [11]).

Previously, we have shown that metastatic lymph nodes contain a proportion (~20%) of cells that simultaneously express the T3, T8, OKM1, and Leu-7 markers [11]. These cells are also present in noninvaded lymph nodes, albeit in
TABLE I

Proliferative Response of Cells from Regional Lymph Nodes to rIL-2

| Stimulus                              | Invaded nodes | Noninvaded nodes |
|---------------------------------------|---------------|------------------|
|                                       | Exp. 1        | Exp. 2 | Exp. 3 | Exp. 1 | Exp. 2 | Exp. 3 |
| Medium alone                          | 250 ± 62      | 246 ± 43 | 405 ± 84 | 498 ± 85 |
| Autologous tumor cells                | 484 ± 58      | 358 ± 96 | 608 ± 101 | 761 ± 113 | 655 ± 98 |
| rIL-2*                                | 5,235 ± 489   | 4,819 ± 898 | 5,434 ± 760 | 5,254 ± 549 | 2,917 ± 518 |
| Autologous tumor cells + rIL-2*       | 8,847 ± 901   | 5,104 ± 478 | 3,866 ± 667 | 5,718 ± 779 | 3,195 ± 555 |

* 2.5 x 10^4 lymph node cells were cultured for 6 d in the presence or absence of 250 irradiated (5,000 rad) autologous tumor cells. Values are expressed as mean ± SD of triplicate cultures.

Data from 3 representative experiments out of 15 performed (8 with bladder, 7 with larynx cancer lymph nodes) are reported.

Data from three representative experiments, out of nine performed (five with bladder, four with larynx cancer lymph nodes), are reported.

rIL-2 was used at 25 U/ml final dilution.

TABLE II

Surface Phenotypes of Cells from Metastatic and Nonmetastatic Lymph Nodes

| Marker | Metastatic nodes (n = 11) | Nonmetastatic nodes (n = 18) |
|--------|---------------------------|-----------------------------|
| T3     | 65.5 ± 11.3               | 54.0 ± 13.9                 |
| B1     | 18.7 ± 3.9                | 29.4 ± 11.2                 |
| Leu-M3 | 9.1 ± 3.7                 | 7.1 ± 2.9                   |
| T4     | 45.2 ± 10.1               | 37.2 ± 9.5                  |
| T8     | 39.8 ± 11.5               | 18.5 ± 6.9                  |
| OKM1   | 27.8 ± 15.3               | 9.8 ± 3.4                   |
| Leu-11 | 4.9 ± 1.8                 | 6.2 ± 2.7                   |
| Leu-7  | 15.3 ± 7.2                | 6.9 ± 5.7                   |
| HLA-DR | 38.9 ± 13.7               | 42.5 ± 10.2                 |
| Tac    | 21.1 ± 8.2                | 11.8 ± 4.2                  |

Mean ± SD of pooled data. Lymph nodes from six patients with carcinoma of the bladder and four patients with carcinoma of the larynx were analyzed.

much lower proportions. These data have been confirmed here (Table II and results of double-staining experiments not shown). Because a number of recent observations have indicated that a subset of human suppressor T cells have a T3+, T8+, OKM1+, Leu-7+ surface phenotype (26, 27), the possibility existed that cells with this phenotype could suppress the in vitro response to malignant cells. Cells from metastatic lymph nodes were depleted of OKM1+, Leu-7+ cells (OKM1−, Leu-7− cells) and tested in MLTC. The results are summarized in Table III. OKM1−, Leu-7− cells were capable of a significant response against malignant cells, provided that exogenous IL-2 was present, i.e., cells cultured with IL-2 and tumor cells proliferated more vigorously than cells cultured with IL-2 alone. Readdition of graded numbers of OKM1+, Leu-7+ cells (up to the proportions occurring in the unfractionated lymph node) inhibited the response to tumor cells in the presence of IL-2, but did not affect the cell proliferation induced by exogenous IL-2. Taken together, these experiments show that
invaded lymph nodes contain a cell population capable of a proliferative response to tumor cells, which can be detected following removal of suppressor cells and addition of exogenous IL-2.

Cells from lymph node with no metastases failed to respond in MLTC even after removal of the OKM1⁺, Leu-7⁺ cells, perhaps suggesting that cells with specificity for malignant cells were absent (data not shown).

Next we investigated whether or not cells from invaded lymph nodes were restricted to respond to autologous cells or could also respond to allogeneic cells. As shown in Table IV, the proliferation of lymph node cells did not increase above the values induced by IL-2 after addition of allogeneic malignant cells. Failure to respond to allogeneic tumor cells was independent of the histologic type of the tumor cells used for stimulation.

The possibility that OKM1⁻, Leu-7⁻ cells could respond in an autologous MLR was investigated by coculturing these cells with autologous peripheral blood non-T cells. A significant response was never observed, even in the presence of exogenous IL-2, thus indicating that invaded lymph nodes contain a very limited number (if any) of T cells reacting in an autologous MLR. These observations exclude the hypothesis that the antigen(s) recognized by OKM1⁻, Leu-7⁻ cells on malignant cells is represented only by autologous MHC-encoded determinants.

Expression of IL-2 Receptors and Failure of Lymph Node Cells to Produce IL-2 in Autologous MLTC. The problem of whether or not coculture with autologous tumor cells could induce IL-2 receptor expression by OKM1⁻, Leu-7⁻ cells was next addressed. OKM1⁻, Leu-7⁻ cells were cocultured with malignant cells for 3 d and subsequently stained for Tac antigen expression. After coculture with autologous tumor cells, a high proportion of OKM1⁻, Leu-7⁻ cells expressed Tac antigen (Fig. 1A). Furthermore, the intensity of staining of the single cells

---

**Table III**

| Stimulus                              | Numbers of OKM1⁺, Leu-7⁺ cells added | [³H]Thiouracil incorporation by OKM1⁻, Leu-7⁻ cells | Exp. 1 | Exp. 2 | Exp. 3 |
|---------------------------------------|-------------------------------------|----------------------------------------------------|-------|-------|-------|
| Medium alone                          | —                                   | 489 ± 27                                           | 327 ± 41 | 521 ± 49 |
| Autologous tumor cells*               | —                                   | 544 ± 46                                           | 389 ± 38 | 589 ± 23 |
| rIL-2*                                | —                                   | 7,249 ± 825                                        | 8,544 ± 1,154 | 7,986 ± 1,084 |
| Autologous tumor cells + rIL-2*       | —                                   | 17,589 ± 1,057                                     | 18,911 ± 2,185 | 17,925 ± 1,825 |
|                                       | 8.0 x 10⁵                            | 8,648 ± 1,115                                      | 8,725 ± 985 | 9,187 ± 1,101 |
|                                       | 4.0 x 10⁵                            | 10,459 ± 988                                       | 11,427 ± 1,529 | 12,185 ± 1,206 |
|                                       | 2.0 x 10⁵                            | 13,728 ± 1,224                                     | 12,689 ± 1,619 | 13,888 ± 1,526 |
|                                       | 1.0 x 10⁵                            | 15,644 ± 1,229                                     | 16,704 ± 1,815 | 17,119 ± 1,614 |

Three representative experiments, two with larynx carcinoma and one with bladder carcinoma lymph node cells, out of six performed, are shown. Mean ± SD of triplicate cultures.

* 250 irradiated (5,000 rad) tumor cells were added.
‡ rIL-2 was added at 25 U/ml final dilution.
TABLE IV
Proliferative Response of OKM1−, Leu-7− Cells from Metastatic Lymph Nodes to Different Cell Types

| Stimulus                                      | [3H]Tdr incorporation by OKM1−, Leu-7− cells |
|-----------------------------------------------|---------------------------------------------|
|                                               | Exp. 1*                                      |
| Medium alone                                  | 389 ± 54                                    |
| rIL-2§                                        | 6,874 ± 1,657                               |
| Allogeneic tumor cells + rIL-2§               |                                             |
| Bladder tumor I                              | 5,352 ± 538                                 |
| Bladder tumor II                             | 6,835 ± 895                                 |
| Larynx tumor I                               | 5,987 ± 1,038                               |
| Larynx tumor II                              | 7,021 ± 973                                 |
| Autologous tumor cells + rIL-2§               | 18,348 ± 2,126                              |
| Autologous non-T cells§                       | 415 ± 171                                   |
| Autologous non-T cells + rIL-2§               | 7,415 ± 526                                 |
| Exp. 2*                                       | 527 ± 79                                    |
| Exp. 3†                                       | 287 ± 44                                    |
|                                              | 8,534 ± 1,421                               |
|                                              | 7,741 ± 1,469                               |
|                                              | 9,719 ± 1,035                               |
|                                              | 7,397 ± 987                                 |
|                                              | 9,562 ± 1,859                               |
|                                              | 8,485 ± 1,024                               |
|                                              | 9,001 ± 1,215                               |
|                                              | 6,984 ± 954                                 |
|                                              | 8,528 ± 938                                 |
|                                              | 7,427 ± 1,045                               |
|                                              | 16,578 ± 1,741                              |
|                                              | 15,291 ± 1,003                              |
|                                              | 398 ± 101                                   |
|                                              | 361 ± 87                                    |
|                                              | 8,144 ± 619                                 |
|                                              | 6,867 ± 489                                 |

Three representative experiments, two with larynx carcinoma and one with bladder carcinoma lymph node cells, out of six performed, are shown. 2.5 × 10⁴ fractionated cells were cultured for 6 h. Mean ± SD of triplicate cultures.

* Fractionated lymph node cells from a larynx cancer patient.
† Fractionated lymph node cells from a urinary bladder cancer patient.
§ rIL-2 was added at 25 U/ml final dilution.
$250 irradiated (5,000 rad) autologous or autologous tumor cells were added.
† 2.5 × 10⁴ irradiated (3,000 rad) autologous peripheral blood non-T cells were added.

increased significantly, as compared to that of Tac+ cells present in the suspension before culture (6–9%) (Fig. 1A). Interestingly, the cells that expressed Tac antigen after 3 d belonged to either the T3+, T4+ or to the T3+, T8+ cell subset (data not shown). By contrast, in the suspensions cultured alone or with autologeneic tumor cells there was no increase in the proportion of Tac+ cells (Fig. 1B). OKM1−, Leu-7− cells did not increase in size when cultured with malignant cells. These findings suggest that the encounter with malignant cells provided a sufficient signal for IL-2 receptor expression, but that the cells could not proceed further in their cycle, probably owing to the absence of IL-2.

To study whether or not the responding cells could produce IL-2 in the MLTC, OKM1−, Leu-7− cells were cultured with malignant cells. IL-2 was measured in the culture supernatants after 3 d. Coculture with either autologous or autologeneic tumor cells failed to induce significant IL-2 production in four consecutive experiments. By contrast, PHA stimulation of the same OKM1−, Leu-7− cells caused abundant IL-2 release (1–2 U/ml per 10⁶ cells).

Characterization of Short-term Lines Derived from MLTC. These experiments were intended to investigate further the fine specificity of OKM1−, Leu-7− cells and to explore the possibility of obtaining cells with cytotoxic properties against tumor cells. As stated above, no cytolytic cells were detected in freshly prepared suspensions.

OKM1−, Leu-7− cells were stimulated with IL-2 and autologous malignant cells, and subsequently expanded by further addition of both IL-2 and malignant cells. With this method, five cell lines were obtained. They comprised cells with either T3+, T8+ or T3+, T4+ phenotype, the former being predominant in four
Figure 1. FACS analysis of Tac antigen expression by cells from metastatic lymph node. Isolated OKM1-, Leu-7- cells were cultured for 72 h in the presence (line 2) or absence (line 3) of malignant cells and stained with anti-Tac mAb. An aliquot of freshly isolated OKM1-, Leu-7- cells from the same cell suspension was stained, formaldehyde-fixed, and analyzed after 3 d in comparison with cultured cells (line 4). Coculture with autologous (A), but not with allogeneic (B), tumor cells induced Tac antigen expression. In this experiment, representative of five performed, the cells had, at day 0, the following surface phenotype: T3+, 71.3%; T4+, 47.5%; T8+, 21.8; HLA-DR+, 26.3%; Tac+, 7.8%. After 3 d, the phenotype was basically unmodified, except that Tac+ cells were 46.3%. Line 1 shows control with FITC-conjugated goat anti-mouse antibodies (GAM Ig) alone. Volume distribution histograms of the same cell populations show no differences in cell size.

The availability of a cell line (FD) that contained predominantly T3+, T8+ cells (i.e., with a cytotoxic cell surface phenotype) allowed to investigate further the question of whether or not a cytotoxic activity could be generated in the system. The T3+, T8+ cell line proliferated specifically in response to autologous but not allogeneic tumor cells in the presence of IL-2 (Table V). Furthermore, the specific proliferative response was abrogated by addition of an anti-HLA-A,B,C mAb and not by anti-HLA-DR mAb. This finding shows restriction of the cells for class I antigens. Interestingly, the treatment with anti-HLA-A,B,C mAb failed to inhibit the response induced by IL-2 alone. The FD cell line showed a weak although definite cytotoxic response against autologous tumor cells. No significant lytic activity was ever observed against three unrelated tumor cells nor to K562 cell line (Fig. 2). The latter control was included to ascertain whether or not the cells possessed an NK-like activity. Specificity for autologous tumor cells was confirmed by cold-target inhibition assays, where autologous but not allogeneic malignant cells inhibited the lytic reaction (Fig. 2).

OKM1-, Leu-7- Cells Can Be Induced to Mount an MLTC by Addition of IL-1. These experiments were intended to investigate the role possibly played by IL-1 in the MLTC response of OKM1-, Leu-7- cells. In the absence of IL-1-
TABLE V

Proliferative Activity of Cell Line FD from Metastatic Lymph Node
OKM1−, Leu-7− Cells

| Stimulus + rIL-2 | [3H]TdR incorporation + rIL-2 |
|------------------|--------------------------------|
| Medium alone     | 1,146 ± 386                   |
| Autologous tumor cells | 1,427 ± 456               |
| Allogeneic tumor cells I | 1,526 ± 712            |
| Allogeneic tumor cells II | 1,289 ± 279           |
| Autologous tumor cells + rIL-2 | 13,784 ± 1,126         |
| Autologous tumor cells + rIL-2 + anti-HLA-A,B,C§ | 21,025 ± 2,284          |
| Autologous tumor cells + rIL-2 + anti-HLA-DR$ | 11,287 ± 995          |
| Allogeneic tumor cells I + rIL-2 | 19,857 ± 2,133         |
| Allogeneic tumor cells II + rIL-2 | 12,825 ± 956          |

Line FD was raised from a larynx cancer patient and comprised 70−80% cells
with a T3+, T8+ surface phenotype. The remaining cells were T3−, T4−.

* Mean ± SD of triplicate cultures. 2.5 × 10⁶ cells were cultured for 3 d.
† Mean ± SD of triplicate cultures. 2.5 × 10⁶ cells were cultured for 3 d.
§ When indicated, 250 irradiated (5,000 rad) autologous or allogeneic (I from a
larynx cancer, II from a urinary bladder cancer) tumor cells and/or rIL-2, 25
U/ml final dilution, were added.
$ Ascites, 1:1,000 final dilution.

Figure 2. Cytotoxic activity of a cell line (FD) derived from OKM1−, Leu-7− cells
isolated from a metastatic lymph node of a larynx cancer patient. Cells were
initially plated in the presence of IL-1 (2 U/ml) and autologous tumor cells, and
subsequently expanded in IL-2-containing medium. After 18 d, the cells were tested
against 51Cr-labeled autologous tumor cells (■), allogeneic larynx cancer cells (○, △), allogeneic bladder cancer cells (□), and K562
cells (○, △) (A). Preincubation with unlabeled autologous (■), but not allogeneic (○, △, □), target
cells caused a marked reduction in the cytotoxic activity against autologous tumor cells (B).

E/T CELL RATIO

% SPECIFIC CYTOTOXICITY

25

20

15

10

5

30:1

10:1

1:1

30:1

10:1

1:1
rich accessory cell supernatants, antigen- or mitogen-activated T cells express IL-2 receptors, but fail to produce IL-2 (28-30). It was therefore possible that the limiting factor for the cells that did not respond in MLTC was represented by the insufficient supply of IL-1. This was also confirmed by the finding that supernatants of 10 different autologous MLTC did not contain detectable IL-1, as assessed in the thymocyte costimulation assay (data not shown). The absence of IL-1 would have in turn impeded both IL-2 production and cell proliferation. To test this hypothesis, OKM1-, Leu-7- cells were cultured with different dilutions of purified or recombinant IL-1, in the presence of malignant cells. Representative experiments with optimal doses of IL-1 are shown in Table VI. Addition of IL-1 resulted in cell proliferation, even in the absence of exogenous IL-2. When IL-1 was added, the cells also produced IL-2. Some proliferation was also induced by IL-1 alone, albeit not comparable to that induced by malignant cells plus IL-1. This phenomenon was not attributable to the mitogenic effect of IL-1, possibly contaminated by endotoxin, as T cells purified from peripheral blood were not induced to proliferate by the same IL-1 preparations, under the same experimental conditions (data not shown). Furthermore, stimulation of lymph node cells by IL-1 was inhabitable in the presence of anti-IL-1 antibodies (Table VI). Rather, lymph node cell proliferation in the absence of malignant cells could be related to the presence of a number of cells already

---

**Table VI**

**Proliferative Response and IL-2 Production by OKM1-, Leu-7- Cells from Metastatic Lymph Nodes in the Presence of IL-1**

| Stimulus | Auto| Anti-IL-1 | [3H]TdR incorporation | IL-2 production |
|----------|-----|-----------|-----------------------|-----------------|
|          | tumor cell | * | Exp. 1 | Exp. 2 | Exp. 1 | Exp. 2 |
| IL-1 |       |       | cpm |       |       | U/ml |       |       |
| 1       | -   | -     | 4,062 ± 587 | 3,529 ± 412 | 0.25 | 0.25 |
|         | +   | -     | 18,445 ± 1,729 | 15,854 ± 918 | 1.75 | 1.50 |
|         | +   | +     | 884 ± 312 | 991 ± 189 | - | - |
|         | -   | +     | 419 ± 98 | 587 ± 121 | - | - |
| 0.5     | -   | -     | 2,144 ± 648 | 1,927 ± 544 | 0.20 | 0.10 |
|         | +   | -     | 16,876 ± 1,385 | 17,442 ± 995 | 1.50 | 1.75 |
|         | +   | +     | 745 ± 227 | 689 ± 212 | - | - |
|         | -   | +     | 527 ± 101 | 581 ± 97 | - | - |
| Allogeneic tumor cells |       |       |       |       |       |       |
| 1       | +   | -     | 4,289 ± 491 | 3,495 ± 567 | 0.25 | 0.20 |
|         | +   | +     | 927 ± 115 | 789 ± 298 | - | - |
| 0.5     | +   | +     | 2,629 ± 881 | 2,129 ± 515 | 0.20 | 0.10 |
|         | +   | +     | 825 ± 191 | 754 ± 253 | - | - |
| Medium alone |       |       | 507 ± 103 | 485 ± 105 | - | - |
|         | +   | +     | 689 ± 81 | 546 ± 75 | - | - |

Data from two representative experiments (one with larynx and one with bladder cancer lymph node cells), out of eight performed, are shown.

* Mean ± SD of triplicate cultures. 2.5 × 10⁶ OKM1-, Leu-7- cells from metastatic lymph nodes were cultured for 6 d.

*OKM1-, Leu-7- cells were cultured at a cell concentration of 10⁶ cells/ml. IL-2 activity was measured in the supernatants by the CTLL-2 proliferation assay.

When indicated, polyclonal rabbit anti-human IL-1 antibodies were added at 1:100 final dilution.

Affinity-purified human monocyte IL-1 was added. Superimposable results were obtained using human rIL-1-d.

When indicated, 250 irradiated (5,000 rad) tumor cells were added.
activated by malignant cells in vivo. Alternatively, the OKM1−, Leu-7− cell suspension could contain contaminant malignant cells capable of providing the appropriate antigenic stimulus.

Discussion

The present study demonstrates that lymph nodes that drain the tumor site contain cells capable of responding to the malignant cells in vitro. These cells have specificity for autologous but not for allogeneic neoplastic cells, as shown by the tests with a panel of allogeneic malignant cells, and could be detected in a proliferative assay only if two conditions were met: (a) exogenous IL-2 must be supplied to sustain the proliferation initiated by coculture with tumor cells, and (b) suppressor cells must be removed. Superimposable results were obtained with two different tumor types, a finding that could suggest a common pattern of immune response shared by different neoplasias.

Infiltrated lymph nodes contained lymphocytes with a T3+, T8+, OKM1+, Leu-7+ surface phenotype that suppressed the response to the malignant cells. The suppressive capacities of T3+, T8+, OKM1+, Leu-7+ lymphocytes were confirmed by the observation that readdition of such cells to the OKM1−, Leu-7− cell suspensions inhibited the response to tumor cells. Suppressor cells have been reported both in mice and humans with malignancies, although different experimental systems have generally been used to detect them (10, 31-34). The mode of action of the suppressor cells described here, together with a characterization of their properties, will be the subject of a separate study. However, it is worth mentioning that the suppression appears to be specific for the response to tumor cells, and is not merely caused by IL-2 consumption by OKM1+, Leu-7+ cells.

Noninvaded lymph nodes contained neither cells responding to the malignant cells nor suppressor cells. These findings may suggest that cells which respond to the malignant cells remain entrapped in the metastatic lymph nodes and do not recirculate.

The present studies were focused on the mechanisms involved in the stimulation of the cells responding to tumor cells. Coculture with autologous tumor cells induced the expression of IL-2 receptors. This step, however, was not followed by cell proliferation because of lack of IL-2 production in the cultures. Recent work on the multistep process of T cell triggering has shown that resting (or memory) and proliferating T lymphocytes require different signals in order to progress through the cell cycle (28, 29, 35-37). In particular, unlike proliferating cells, resting T lymphocytes require, in addition to the presence of antigen, a signal delivered by IL-1 (28, 29, 38). It is still debated whether or not this signal is necessary for the subsequent expression of IL-2 receptors (39, 40), or whether it induces the production of IL-2 (28, 29). The present data are in agreement with the latter hypothesis. In the absence of detectable IL-1, T cells expressed IL-2 receptors after antigen stimulation, but began to produce IL-2 only after the addition of exogenous IL-1. While the absence of IL-1 production

2 Cozzolino, F., M. Torcia, A. M. Carossino, R. Giordani, C. Selli, G. Talini, E. Reali, A. Novelli, V. Pistoia, and M. Ferrari. Functional analysis of suppressor cells from regional lymph nodes of cancer patients. Manuscript in preparation.
explains the failure of T cells to produce IL-2 in the MLTC, there are no clear explanations for the lack of IL-1 production after stimulation with malignant cells. One possible interpretation could be that macrophages or other accessory cells committed to IL-1 production are not induced to do so solely by the presence of (or encounter with) neoplastic cells. An alternative and not mutually exclusive explanation may be that T cells have to activate accessory cells to release IL-1. This activation may be mediated, for example, through the production of IFN-γ (41, 42). The recognition of tumor cells may not be a sufficient signal for inducing the release of this lymphokine. The possibility also exists that macrophages from the metastatic lymph nodes have an impaired capacity of producing IL-1, as reported in other experimental systems (43). Finally, the possible presence of IL-1 inhibitors has to be considered (44). All of the above hypotheses are compatible with the observation that OKM1−, Leu-7− cells respond to PHA. The response to this mitogen is less dependent on accessory cells, and PHA per se induces abundant IL-1 release by macrophages (45).

The short-term lines derived from the cells responding to tumor cells required the constant presence of IL-2 for antigen-induced proliferation. This observation raises the question of whether or not they represent a special T cell subset. However, so far, the surface marker analysis has not revealed particular differences between cells responding to tumor cells and those directed to other types of antigen. Furthermore, cells that are dependent on constant stimulation with both antigen and IL-2 for growth have been described also in different experimental systems (46–49).

T cells isolated from lymph nodes were not cytotoxic for the malignant cells when tested immediately after isolation. Cytotoxic T cells were, however, detected in one of the cell lines obtained. The availability of this cell line provided a further opportunity for testing both the antigen specificity and the restriction of the responding cells. Again, these cells were induced to proliferate only by the autologous malignant cells. Furthermore, their proliferative response was inhibited by an anti-HLA-A,B,C mAb, as could be expected for class I–restricted cytolytic T cells. Finally, the lytic activity was exerted only and specifically on the autologous malignant targets. Taken together, these data indicate that lymph nodes from patients with malignancies contain cytotoxic T cell precursors, which may be usable in novel therapeutical approaches.

Summary
The specific immune response against the malignant cells was investigated in patients with urinary bladder or larynx cancer. Lymphocytes from lymph nodes that drain the tumor site were tested for their proliferative and cytotoxic capacities against autologous malignant cells isolated from the primary tumor. In no occasion was a proliferative or a cytotoxic response observed. However, when the lymph node cell suspensions were depleted of cells expressing both OKM1 and Leu-7 markers by rosetting with the appropriate mAbs, a proliferative response could be observed. The lymphocytes responded to autologous tumor cells only if IL-2 was added to the cultures. IL-2 alone induced some cell proliferation, which was not, however, comparable to that observed in response to both IL-2 and tumor cells. A panel of allogeneic tumor cells consistently failed
to stimulate OKM1−, Leu-7− cells in vitro. Response to autologous tumor cells was not caused by HLA-encoded molecules, as occurs in the autologous mixed lymphocyte reaction, since OKM1−, Leu-7− cells failed to be stimulated by autologous non-T cells. A proliferative response was observed only with cells from lymph nodes that had been classified as invaded by malignant cells according to histopathologic criteria. Cells from noninvaded lymph nodes consistently failed to respond.

Cells stimulated with autologous tumor cells could be expanded in short-term lines by continuous addition of IL-2 and malignant cells. One of these lines, which comprised mainly T8+ cells, was stimulated to proliferate only by autologous tumor cells, and its proliferative response was inhabitable by anti-class I and not by anti-class II mAbs. This line showed lytic capacities against autologous malignant targets, while it was inefficient against all of the other allogeneic cells tested.

In another set of experiments, the mechanisms whereby exogenous IL-2 had to be added to the cultures to sustain a proliferative response against neoplastic cells were investigated. When cocultured with autologous malignant cells, OKM1−, Leu-7− lymphocytes expressed IL-2 receptors, as could be assessed by anti-Tac fluorescent staining. Under these culture conditions, these cells did not produce IL-2, and no proliferation was observed. Addition of purified IL-1 to the cultures induced IL-2 production and cell proliferation.

It is concluded that metastatic lymph nodes contain a T cell population that can be detected in a proliferative assay when both suppressor cells are removed and the appropriate molecular signals are supplied.

We thank Drs. C. E. Grossi, N. Chiorazzi, G. Forni, and A. Mantovani for helpful suggestions and for critically reviewing this manuscript. We also thank Drs. T. A. Waldmann and G. Damiani for providing the anti-Tac, PTF 29/12, and 22.1 mAbs. We are grateful to Biogen S.A., Geneva, Switzerland, for the generous supply of rIL-2 and rIL-1-β.

Received for publication 29 October 1986 and in revised form 16 March 1987.

References

1. North, R. J. 1984. The murine anti-tumor response and its therapeutic manipulation. Adv. Immunol. 35:89.
2. Schreiber, H. 1984. Idiotype network interaction in tumor immunity. Adv. Cancer Res. 41:291.
3. Drebin, J. A., L. L. Perry, R. Carter, and M. I. Greene. 1982. Regulation of the immune response to antigens on the malignant cell surface. Springer Semin. Immunopathol. 5:175.
4. Serrate, S. A., B. Vose, T. Timonen, J. R. Ortaido, and R. B. Herberman. 1982. Association of human natural killer cell activity against primary tumors with large granular lymphocytes. In NK and Other Natural Effector Cells. R. B. Herberman, editor. Academic Press, Inc., New York, p. 1055.
5. Masucci, M. G., M. T. Bejarano, F. Vanky, and E. Klein. 1982. Cytotoxic and cytostatic activity of human large granular lymphocytes against allogeneic tumor biopsy cells and autologous EBV infected B lymphocytes. In NK and Other Natural Effector Cells. R. B. Herberman, editor. Academic Press, Inc., New York, p. 1047.
TUMOR-SPECIFIC LYMPHOCYTE RESPONSE

6. Rosenberg, S. A. 1985. Lymphokine-activated killer cells. A new approach to immunotherapy of cancer. J. Natl. Cancer Inst. 75:595.

7. Johnson, W. J., S. T. Somers, and D. O. Adams. 1984. Expression and development of macrophage activation for tumor cytotoxicity. Contemp. Top. Immunobiol. 15:127.

8. Uchida, A., and M. Mick. 1982. Autologous mixed lymphocyte reaction in the peripheral blood and pleural effusions of cancer patients. J. Clin. Invest. 70:98.

9. Ferrini, S., R. Biassoni, A. Moretta, M. Bruzzone, A. Nicolini, and L. Moretta. 1985. Clonal analysis of T lymphocytes isolated from ovarian carcinoma ascitic fluid. Phenotypic and functional characterization of T-cell clones capable of lysing autologous carcinoma cells. Int. J. Cancer. 36:337.

10. Mukherji, B., S. A. Wilhelm, A. Guha, and M. T. Ergin. 1986. Regulation of cellular immune response against autologous human melanoma. I. Evidence for cell mediated suppression of in vitro cytotoxic immune response. J. Immunol. 136:1888.

11. Cozzolino, F., M. Torcia, E. Castigli, C. Selli, R. Giordani, A. M. Carossino, M. Squadrelle, M. Cagnoni, V. Pistoia, and M. Ferrarini. 1986. Presence of activated T cells with a T8+ M1+ Leu 7+ surface phenotype in invaded lymph nodes from patients with solid tumors. J. Natl. Cancer Inst. 77:637.

12. Reinherz, E. L., and S. F. Schlossman. 1980. The differentiation and function of human T lymphocytes: a review. Cell. 19:821.

13. Sanchez-Madrid, F., J. A. Nagy, E. Robbins, P. Simon, and T. A. Springer. 1983. A human leukocyte differentiation antigen family with distinct a-subunits and a common b-subunit: the lymphocyte function-associated antigen (LFA-1), the C3bi complement receptor (OKM1/Mac-1), and the p-150,95 molecule. J. Exp. Med. 158:1785.

14. Dimitriu-Bona, A., G. R. Burmeister, S. J. Waters, and R. J. Winchester. 1983. Human mononuclear phagocyte differentiation antigens. 1. Patterns of antigenic expression on the surface of human monocytes and macrophages defined by monoclonal antibodies. J. Immunol. 130:145.

15. Phillips, J. H., and G. F. Babcock. 1983. NKP-15: a monoclonal antibody reactive against purified human natural killer cell and granulocytes. Immunol. Lett. 6:143.

16. Stashenko, P., L. M. Nadler, R. Hardy, and S. F. Schlossman. 1980. Characterization of a human B lymphocyte specific antigen. J. Immunol. 125:1678.

17. Abo, T., and C. M. Balch. 1981. A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). J. Immunol. 127:1024.

18. Abo, T., M. D. Cooper, and C. M. Balch. 1982. Postnatal expansion of the natural killer and killer cell populations in humans identified by the monoclonal HNK-1 antibody. J. Exp. Med. 155:321.

19. Uchiyama, T., S. Broder, and T. A. Waldmann. 1981. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. 1. Production of anti-Tac monoclonal antibody and distribution of Tac+ cells. J. Immunol. 126:1393.

20. Corte, G., G. Damiani, F. Calabi, M. Fabb, and A. Bargellesi. 1981. Analysis of HLA-DR polymorphism by two-dimensional peptide mapping. Proc. Natl. Acad. Sci. USA. 78:534.

21. Damiani, G., F. Zocchi, M. Fabb, O. Secco, F. Patrone, G. A. Rossi, and A. Bargellesi. 1985. Non-DR molecules present on monocytes participate in the development of A-MLR in humans. In Biotechnology in Diagnostic. H. Koprowski, S. Ferrone, and A. Albertini, editors. Elsevier Science Publishers, New York. p. 57.

22. Ferrarini, M., A. Bargellesi, G. Corte, G. Viale, and B. Pernis. 1975. Comparative study of membrane and intracytoplasmic Ig classes in human lymphoid cells. Ann. NY Acad. Sci. 254:243.

23. Pistoia, V., F. Cozzolino, M. Torcia, E. Castigli, and M. Ferrarini. 1985. Production
of B cell growth factor by a Leu-7+, OKM1+ non-T cell with the features of large granular lymphocytes (LGL). J. Immunol. 134:3179.

24. Egeland, T., and T. Lea. 1982. A rapid rosette technique for quantitation and separation of mononuclear cell subsets using monoclonal antibodies. J. Immunol. Methods. 55:213.

25. Kedar, E., B. L. Ikejiri, G. D. Bonnard, and R. B. Herberman. 1982. A rapid technique for isolation of viable tumor cells from solid tumors: use of the tumor cells for induction and measurement of cell-mediated cytotoxic responses. Eur. J. Cancer Clin. Oncol. 18:991.

26. Landay, A., G. L. Garland, and L. T. Clement. 1983. Characterization of a phenotypically distinct subpopulation of Leu 2+ cells that suppresses T cell proliferative responses. J. Immunol. 131:2757.

27. Clement, L. T., C. E. Grossi, and G. L. Garland. 1984. Morphological and phenotypic features of the subpopulation of Leu 2+ cells that suppresses B cell differentiation. J. Immunol. 133:2471.

28. Manger, B., A. Weiss, C. Weyand, J. Goronzy, and J. D. Stobo. 1985. T cell activation: Differences in the signals required for IL 2 production by non-activated and activated T cells. J. Immunol. 135:3669.

29. Meuer, S. C., and K. H. Meyer zum Büschenfelde. 1986. T cell receptor triggering induces responsiveness to Interleukin 1 and Interleukin 2 but does not lead to T cell proliferation. J. Immunol. 136:4106.

30. Tsoukas, C. D., B. Landgraf, J. Bentin, M. Valentine, M. Lotz, J. H. Vaughan, and D. A. Carson. 1985. Activation of resting T lymphocytes by anti-CD3 (T3) antibodies in the absence of monocytes. J. Immunol. 135:1719.

31. Mills, C. D., and R. J. North. 1983. Expression of passively transferred immunity against an established tumor depends on generation of cytolytic T cells in recipient. Inhibition by suppressor T cells. J. Exp. Med. 157:1448.

32. North, R. J., and I. Bursuker. 1984. Generation and decay of the immune response to a progressive fibrosarcoma. I. Ly-1+, 2+ suppressor T cells downregulate the generation of Ly-1-, 2+ effector T cells. J. Exp. Med. 159:1295.

33. Flood, P. M., M. L. Kripke, D. A. Rowley, and H. Schreiber. 1980. Suppression of tumor rejection by autologous antidiotype immunity. Proc. Natl. Acad. Sci. USA. 77:2209.

34. Mukherji, B. A. L. Nashed, A. Guha, and M. T. Ergin. 1986. Regulation of cellular immune response against autologous human melanoma. II. Mechanism of induction and specificity of suppression. J. Immunol. 136:1893.

35. Austyn, J. M., R. M. Steinman, D. E. Weinstein, A. Granelli-Piperno, and M. A. Palladino. 1983. Dendritic cells initiate a two-stage mechanism for T lymphocyte proliferation. J. Exp. Med. 157:1101.

36. Inaba, K., and R. M. Steinman. 1984. Resting and sensitized T lymphocytes exhibit distinct stimulatory (antigen-presenting cell) requirements for growth and lymphocyte release. J. Exp. Med. 160:1717.

37. Meuer, S. C., R. E. Hussey, D. A. Cantrell, J. C. Hodgdon, S. F. Schlossman, K. A. Smith, and E. L. Reinherz. 1984. Triggering of the T3-Ti antigen-receptor complex results in clonal T cell proliferation through an interleukin-2-dependent autocrine pathway. Proc. Natl. Acad. Sci. USA. 81:1509.

38. Depper, J. M., W. J. Leonard, M. Krönke, P. D. Noguchi, R. E. Cunningham, T. A. Waldmann, and W. C. Greene. 1984. Regulation of Interleukin 2 receptor expression: effects of phorbol diester, phospholipase C, and re-exposure to lectin or antigen. J. Immunol. 133:3054.

39. Williams, J. M., D. Deloria, J. A. Hansen, C. A. Dinarello, R. Loertscher, H. M.
Shapiro, and T. B. Strom. 1985. The events of primary T cell activation can be staged by use of Sepharose-bound anti T3 (64.1) monoclonal antibody and purified interleukin 1. J. Immunol. 135:2249.

40. Schwab, R., M. K. Crow, C. Russo, and M. Weksler. 1985. Requirements for T cell activation by OK T3 monoclonal antibody: role of modulation of T3 molecules and Interleukin 1. J. Immunol. 135:1714.

41. Boraschi, D., S. Censini, and A. Tagliabue. 1984. Interferon-γ reduces macrophage-suppressor activity by inhibiting prostaglandin E2 release and inducing Interleukin 1 production. J. Immunol. 133:764.

42. Arenzana-Seisdedos, F., J. L. Virelizier, and W. Fiers. 1985. Interferons as macrophage-activating factors. III. Preferential effect of Interferon-γ on the Interleukin 1 secretory potential of fresh or aged human monocytes. J. Immunol. 134:2444.

43. Peri, G., V. Rossi, G. Taraboletti, A. Erroi, and A. Mantovani. 1986. Ia antigen expression and IL 1 production in murine tumor-associated macrophages. Immunology. 59:527.

44. Roberts, N. J., Jr., A. H. Prill, and T. N. Mann. 1986. Interleukin 1 and interleukin 1 inhibitor production by human macrophages exposed to influenza virus or respiratory syncytial virus. Respiratory syncytial virus is a potent inducer of inhibitor activity. J. Exp. Med. 163:511.

45. Bayne, E. K., E. A. Rupp, G. Limjuco, J. Chin, and J. A. Schmidt. 1986. Immunocytochemical detection of interleukin 1 within stimulated human monocytes. J. Exp. Med. 163:1267.

46. Lowenthal, J. W., C. Tougne, H. R. MacDonald, K. A. Smith, and M. Nabholz. 1985. Antigenic stimulation regulates the expression of IL 2 receptors in a cytolytic T lymphocyte clone. J. Immunol. 134:931.

47. Andrew, M. E., and T. J. Braciale. 1981. Antigen-dependent proliferation of cloned continuous lines of H-2-restricted influenza virus-specific cytotoxic T lymphocytes. J. Immunol. 127:1201.

48. Raulet, D. H., and M. J. Bevan. 1982. Helper T cells for cytotoxic T lymphocytes need not be I region restricted. J. Exp. Med. 155:1766.

49. Albert, F., M. Buferne, C. Boyer, and A. M. Schmitt-Verhulst. 1982. Interaction between MHC-encoded products and cloned T-cells. I. Fine specificity of induction of proliferation and lysis. Immunogenetics. 16:533.