Original Article

J Chin Med Assoc
2004;67:323-330

Interleukin-2 Stimulation Activates Mesothelial Cellular Functioning Against Autologous Tumor Cells

Yuh-Min Chen
Yi-Lin Hsieh
Chun-Ming Tsai
Reury-Perng Perng

Chest Department, Taipei Veterans General Hospital and National Yang-Ming University School of Medicine, Taipei, Taiwan, R.O.C.

Key Words
interleukin-2;
interleukin-12;
interferons;
pleural effusion, malignant

Background. The present study was designed to determine the different effects of cytokines or antibodies (IL-2, IL-4, IL-7, IL-10, IL-12, αCD3) in stimulating the cellular functions of mesothelial cells isolated from malignant pleural effusion.

Methods. Mesothelial cells were isolated from 27 patients with malignant pleural effusion. The cultured cellular interferon-γ (IFNγ) and IL-10 production, proliferative response, and cytolytic activity against autologous tumors and K-562 cells were measured.

Results. Stimulation with IL-2 alone significantly increased the mesothelial cells' proliferative response ($p < 0.001$) and cytolytic activity against autologous tumors ($p = 0.025$). The further addition of other cytokines did not increase these functions. The IFNγ/IL-10 ratio data showed that the T-helper (Th) pathway was shifted from the Th-2 pathway to the Th-1 pathway (increase of IFNγ/IL-10 ratio) when mesothelial cells were stimulated with IL-2. Further stimulation with IL-2 plus IL-12 or αCD3 shifted the Th pathway further in the Th-1 direction, but without statistical significance.

Conclusions. The mesothelial cell proliferative response is enhanced with IL-2 stimulation alone. The T-helper pathway is also shifted from the Th-2 to the Th-1 response (increase of IFNγ/IL-10 ratio) after IL-2 stimulation of mesothelial cells. Mesothelial cells had cytolytic activity against tumor cells, and this activity could be augmented by IL-2 stimulation.

Mesothelial cells form monolayers that line the body cavities, such as the pleural cavity (parietal pleura) and the outer surfaces of the organs within (visceral pleura covering the lungs). The mesothelium is involved in the transport of fluids and solutes, in both directions, between the circulation and the body cavity, as was evident in patients receiving peritoneal dialysis.[1] During bacterial infection of the pleura cavity (empyema), the mesothelium is responsible for the initiation of the inflammatory response by phagocytosis, and the releasing of a lot of chemokines and cytokines to attract and transport neutrophils from the systemic circulation and through the mesothelium.[2-9] Mesothelial cells are closely related to endothelial cells; both are effective presenters of antigens to specific T-helper lymphocytes.[10]

An accumulation of mesothelial cells, lymphocytes, macrophages, and tumor cells in the pleural fluid has been found frequently in malignant effusions secondary to pleural involvement and/or metastases by malignancies.[11,12] It was noted that mesothelial cells were abundant in the early phase of the formation of malignant pleural effusion. However, the mesothelial cells decreased in number as the tumor burden increased in the effusions, and finally disappeared in the effusions with only tumor cells left.[11,12] The role of mesothelial cells in the local cellular immune reaction against cancer cells was much less clear than their role in the immune reaction against bacterial infection.[2-10]

In our previous studies, we found there was a significant elevation of interleukin-10 (IL-10), in favor of the T-helper (Th) 2 pathway, in malignant pleural effusion when compared with peripheral blood levels.[13] Lymphocytes isolated from malignant pleural effusion were in an immunosuppressed status, and could be re-

Received: October 21, 2003.
Accepted: May 13, 2004.

Correspondence to: Dr. Yuh-Min Chen, Chest Department, Taipei Veterans General Hospital, 201, Sec. 2, Shih-Pai Road, Taipei 112, Taiwan.
Tel: +886-2-2876-3466; Fax: +886-2-2875-2380; E-mail: ymchen@vghtpe.gov.tw

activated by two-signal stimulations.[14-16] In spite of these studies, we still could not identify the role of mesothelial cells in the local tumor immune reaction of the pleural cavity.

In the present study, we used mesothelial cells isolated from malignant pleural effusion to study the different effects of IL-2, IL-4, IL-7, IL-10, IL-12, and/or αCD3 on the stimulation or suppression of the cellular functions of mesothelial cells, including cytokine production in relation to the Th pathway (interferon-γ, IL-10), cell proliferation, and cytolytic activity against tumor cells.

METHODS

Patient population

Pleural effusion samples were collected from 27 newly-diagnosed cancer patients with malignant pleural effusion. All patients had had a positive effusion cytology examination for malignant cells, and included 22 patients with pulmonary adenocarcinoma, 4 with poorly differentiated carcinoma of the lung, and 1 with adenocarcinoma of the breast. None of the patients had received anti-cancer treatment, corticosteroid, or other non-steroid anti-inflammatory drugs within 1 month before sample collections.

Preparation of effusions

Pleural effusion specimens were collected in acid citrate dextrose (ACD solution-C) bottles to prevent clotting. The effusion was immediately centrifuged, and the cell pellet was resuspended in RPMI 1640 (Whittaker Bioproducts, Walkersville, MD, USA), and then layered on Ficoll-Hypaque (Amersham Biosciences, Uppsala, Sweden) cushions. After centrifugation, the tumor cells and mononuclear cells were collected from the interface and washed twice in RPMI 1640. Tumor cells were then separated from mononuclear cells by centrifugation on a discontinuous Percoll density gradient.

Separation of mesothelial cells from tumor cells

The tissue culture medium (TCM) contained RPMI 1640 supplemented with 10% fetal bovine serum (GIBCO, Grand Island Biological Co., Grand Island, NY, USA), 2 mM glutamine (GIBCO), 50 μg/mL streptomycin, and 500 units/mL penicillin. Percoll (Amersham Biosciences, Uppsala, Sweden) was adjusted to 20% and 40% in the TCM. Cells from pleural effusion were resuspended in 20% Percoll and layered on 40% Percoll, then centrifuged at 312 × g for 60 min at 25°. After centrifugation, 3 layers of cells were noted. Cells from each band were then collected, washed, and resuspended in TCM. Cells from the upper band of the effusions were 90-98% tumor cells; they were washed and subsequently cultured for use as autologous tumor target cells. Cells from the middle band of the effusions were mainly mesothelial cells, with less than 15% mononuclear cells and/or tumor cells each. Cells from the bottom band consisted of more than 98% mononuclear cells, with less than 1-2% tumor cells or mesothelial cells, and some red cells. Cells from the middle band were washed with TCM twice and served as mesothelial cells for culturing in different conditions. The cell viability was greater than 95%, as determined by trypan blue dye exclusion.

Mesothelial cell culture

Cells from fraction 2 were mainly mesothelial cells and were resuspended at 4×10^5 cells/mL in TCM, and allowed to adhere in plastic flasks overnight. The non-adherent fraction was removed, and the adherent fraction (enriched for mesothelial cells) was allowed to grow in TCM. Macrophages were diluted out with subsequent passaging of confluent cultures; tumor cell outgrowth is rare under these conditions.[10] After 2 passages, the cells were harvested and used as mesothelial cells for culturing in different conditions. Mesothelial cells were confirmed by cytologic examinations of the harvested cells.

Tumor cell culture

Tumor cells were cultured in ACL-4 medium supplemented with 5% fetal bovine serum (GIBCO), 2 mM glutamine (GIBCO), 50 μg/mL streptomycin, and 500 units/mL penicillin. After one or 2 passages, they were harvested and used as autologous tumor cell targets.

Cytokines and monoclonal antibodies (MoAb)

Purified human IL-2, IL-4, IL-7, IL-10, and IL-12 were purchased from R & D Systems, Inc., (Minneapolis, MN, USA). The αCD3 Ab was produced in hybridoma

OKT3, and the culture supernatant was used.[17]

Activation of effectors

Mesothelial cells were incubated at 4×10^5 cells/mL in TCM alone or with the different concentrations of cytokines mentioned above and/or αCD3. A cell proliferation assay was performed after 3 days of culture at 37 °C in a CO_2 incubator. Cell-mediated cytotoxicity, and interferon-γ (IFNγ) and IL-10 production by the mesothelial cells, were determined after 6 days of culture at 37 °C in a CO_2 incubator.

ELISA analysis of IFNγ and IL-10 levels

The 6-day cultured media of the various groups were centrifuged at 4 °C, and supernatants were collected and stored at -70 °C in aliquots of 0.25 mL in microcentrifuge tubes. IFNγ and IL-10 were determined in duplicate by employing the ELISA kit (Quantikine, R & D System, Inc., Minneapolis, MN, USA) for a solid-phase ELISA method that employed the quantitative "sandwich" enzyme immunoassay technique. Positive and negative controls were included in the assay.

Cell proliferation assay

The effects of different treatments on the proliferation of activated mesothelial cells were determined by a ^{3}H-thymidine incorporation assay. In brief, 0.2 mL of mesothelial cells was cultured (alone or incubated with cytokines) directly in 96-well flat-bottom microplates in triplicate, and the cells were incubated at 37 °C in a CO_2 incubator for 3 days. Then, ^{3}H-thymidine at 2 μCi/mL was added to each well and incubated overnight. The incorporation of ^{3}H was determined by a Beta-plate counter, with results expressed as CPM ± SEM.

Cell-mediated cytotoxicity assay

Overnight ^{51}Cr release assay was used to detect the cell-mediated cytotoxicity. The results were expressed as total percentage of lysis and net percentage of lysis, according to the following formulas:

$$\text{Total \% lysis} = \frac{\text{cpm in supernatant}}{\text{total cpm}} \times 100$$

Net % lysis = (total % lysis of the test effectors) - (total % lysis of the medium control).

Statistical analysis

The Wilcoxon signed ranks test was used to determine whether or not there existed a significant difference ($p < 0.05$) in cytokine production, proliferative activity, and cytolytic activity among the different culture conditions.

RESULTS

IL-2 stimulation alone induced the best proliferative response of mesothelial cells

Twenty-three samples of freshly isolated mesothelial cells from 27 patients with malignant pleural effusion received a proliferative examination on day 4 of culturing. The proliferation elicited by IL-2, IL-4, IL-7, IL-10, IL-12, and αCD3 on these freshly isolated mesothelial cells is depicted in Table 1 and Fig. 1. A significantly higher proliferative response was achieved with IL-2, IL-7, or αCD3 stimulation alone. IL-4, IL-10, or IL-12 alone was useless in stimulating the proliferative response of the mesothelial cells. This proliferative response was highest with IL-2 stimulation. Next, the combined effect of IL-2 with other cytokines on the proliferative response was also examined, and revealed no further enhancement of proliferation, even depressing it as compared to an IL-2 culture alone (Fig. 1).

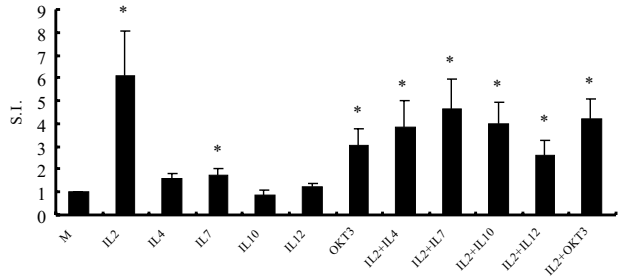

Fig. 1. Proliferation elicited by IL-2, IL-4, IL-7, IL-10, IL-12, or αCD3 on freshly isolated mesothelial cells. Freshly isolated mesothelial cells (4×10^5/mL) were cultured with IL-2 150 IU/mL, IL-4 10 ng/mL, IL-7 10 ng/mL, IL-10 10 ng/mL, IL-12 10 ng/mL, αCD3 1/100 dilution, or IL-2 in combination with other cytokines. A proliferation assay was performed on day 3. A significantly higher proliferative response was achieved with IL-2, IL-7 and αCD3 (*$p < 0.01$, compared with mesothelial cells alone). S.I.: stimulation index (mean cpm of test samples/mean cpm of mesothelial cell culture alone). The mean value of the cpm of the mesothelial cell culture alone in 23 samples was 1090.

IL-2 stimulation shifted the Th pathway from Th-2 to Th-1

The ratio of IFNγ/IL-10 could be used as an indicator of the T-helper (Th) pathway. The supernatants of 7 cases of mesothelial cells cultured under different cytokine stimulations were collected for IFNγ and IL-10 production analysis. The results showed that the Th pathway was shifted from the Th-2 pathway toward the Th-1 pathway (increasing the ratio of IFNγ/IL-10) in the presence of IL-2, IL-12, or αCD3. IL-4, IL-7, and IL-10 were useless in the stimulation of the Th-1 pathway. Further stimulation with IL-2 plus IL-12, or IL-2 plus αCD3 shifted the Th pathway more in the Th-1 direction, but without statistical significance (Table 2).

IL-2 stimulation alone enhanced mesothelial cell cytolytic activity against autologous tumor cells

Among the 27 study cases, only 8 cases had adequate mesothelial cells and autologous tumor cells for cytotoxic studies. The cell samples underwent a cytotoxic test after

Table 1. Proliferative response of mesothelial cells to different stimulations (n = 23)

S.I.	Mean	SEM	Range	*p* value*	*p* value#
M	1.00	0.00	1.00	-	-
M + IL2 150 U/mL	6.13	1.92	1-41.9	< 0.001	-
M + IL4 10 ng/mL	1.57	0.25	0.8-3.01	0.109	< 0.001
M + IL7 10 ng/mL	1.76	0.27	0.97-3.4	0.011	0.008
M + IL10 10 ng/mL	0.88	0.22	0.3-1.6	0.416	< 0.001
M + IL12 10 ng/mL	1.25	0.14	0.3-2.5	0.121	< 0.001
M + αCD3 (1/100 dilution)	3.07	0.69	0.1-12	0.006	0.112
M + IL2 + IL4	3.88	1.11	0.8-10.2	0.011	0.011
M + IL2 + IL7	4.67	1.28	1.48-11.8	0.008	0.173
M + IL2 + IL10	4.02	0.92	2.01-8.2	0.028	0.249
M + IL2 + IL12	2.63	0.63	0.2-14.57	0.002	< 0.001
M + IL2 + αCD3	4.22	0.87	0.1-14.3	0.006	0.248

Abbreviations: S.I. = stimulation index (mean cpm of test samples/mean cpm of mesothelial cell culture alone); M = mesothelial cell; SEM = standard error of the mean.

* Compared with the mesothelial cell culture alone.

Compared with the mesothelial cell + IL2 culture.

Table 2. IFNr/IL10 ratio of mesothelial cells stimulated by different cytokines (n = 7)

	Mean	SEM	Range	*p* value*	*p* value#
M	1.21	0.93	0-6.7	-	-
M + IL2 150 U/mL	35.63	29.85	0.03-214.5	0.028	-
M + IL4 10 ng/mL	0.81	0.49	0-2.8	0.461	0.018
M + IL7 10 ng/mL	1.86	1.42	0-10.1	0.461	0.018
M + IL10 10 ng/mL	0.007	0.005	0-0.04	0.068	0.018
M + IL12 10 ng/mL	32.84	12.50	2.3-83.8	0.018	0.498
M + αCD3 (1/100 dilution)	15.07	4.14	0.8-31	0.028	0.612
M + IL2 + IL4	3.09	1.95	0-14.5	0.500	0.176
M + IL2 + IL7	5.84	2.52	0-19.1	0.028	0.499
M + IL2 + IL10	0.83	0.45	0-2.1	1.000	0.068
M + IL2 + IL12	173.73	116.40	4.2-852.2	0.018	0.128
M + IL2 + αCD3	81.73	58.14	0.2-426.6	0.018	0.063

Abbreviations: M = mesothelial cell; SEM = standard error of the mean.

* Compared with the mesothelial cell culture alone.

Compared with the mesothelial cell + IL2 culture.

culturing under different conditions. Cytolytic activity against autologous tumors and K-562 cells was tested after culturing with IL-2 with/without IL-12 or αCD3. The majority of the test samples showed an autologous tumor cell-killing effect in proportion to E:T ratios; the higher the ratio the higher the killing effect, at E:T ratios of 50:1, 15:1, 5:1, or 15:1, 4.5:1, 1.5:1. The cytolytic effect against K-562 cells was relatively lower and inconsistent. A representive case is shown in Fig. 2 (Panel A). The addition of IL-2 significantly increased the mesothelial cells' cytolytic activity against autologous tumors cells ($p = 0.025$), but not against K-562 cells ($p = 0.249$) (Fig. 2, Panel B). The cytolytic activity of the mesothelial cells was also increased in the presence of IL-12 or αCD3 stimulation, but not to statistical significance (Table 3). Cytolytic activity against autologous tumors was not further significantly increased when αCD3 or IL-12 was added in addition to IL-2 (Fig. 2).

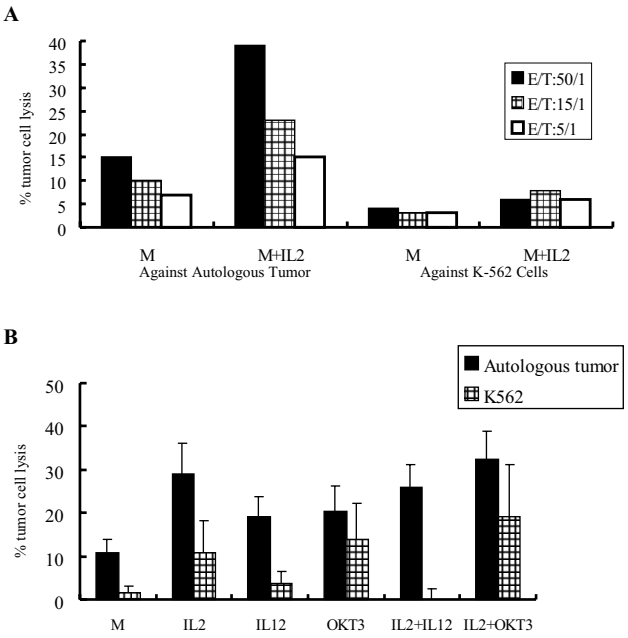

Fig. 2. Mesothelial cells' cytolytic activity was restored by IL-2 stimulation. The results were obtained by testing 8 individual patients. The mesothelial cells were cultured with IL-2 150 IU/mL, with/without IL-12 10 ng/mL or αCD3 1/100 dilution, and 6-day cultured cells were tested against the autologous tumor target and K562 cells. *Panel A.* A representative case with cytolytic activity expressed as % lysis at an effector/target ratio of 50/1, 15/1, and 5/1 is shown. *Panel B.* Cytolytic activity is expressed as % lysis at an effector/target ratio of 15/1. *$p = 0.025$, compared with the mesothelial cell culture alone.

DISCUSSION

The use of immunostimulatory cytokines has become an increasingly promising approach in cancer immunotherapy. The major goal of immunotherapy is the activation of tumor-specific killer cells capable of rejecting tumor cells in cancer patients. IL-2, IL-4, IL-7, IL-10, and IL-12 have been used to promote the generation of LAK cells, to stimulate CTL development, or to induce tumor rejection or reduce metastases.[16]

It has been found that mesothelial cells can produce inflammatory cytokines (G-CSF, GM-CSF, IL-1, and IL-6) in malignant effusion.[18] In addition, an intraperitoneal injection of genetically modified human mesothelial cells, as a type of systemic gene therapy, has been used in an athymic mice model to produce human growth factor.[19] However, whether or not mesothelial cells can be

Table 3. Cytolytic activity of mesothelial cells stimulated by different cytokines (n = 8)

% lysis*	Autologous tumor				K562 cells			
	Mean	Range	SEM	*p* value#	Mean	Range	SEM	*p* value#
M	10.85	0-20	3.02	-	1.41	0-7.8	1.56	-
M + IL2	28.94	3.5-63.9	7.14	0.025	10.70	0-46.6	7.63	0.249
M + IL12	19.22	5-37.8	4.56	0.128	3.74	0-14.1	2.84	0.600
M + OKT3	20.31	2-45.1	5.88	0.310	13.91	0-53.5	8.43	0.249
M + IL2 + IL12	25.82	7-50.8	5.21	0.092	0.06	3-18.3	2.34	0.116
M + IL2 + OKT3	32.48	14-70.5	6.27	0.612	19.22	0-76.1	11.94	0.249

Abbreviations: M = mesothelial cell; SEM = standard error of the mean.

*At effector/target ratio of 15/1.

#Compared with the mesothelial cell alone.

used in immunotherapy against autologous tumor cells is still unknown.

Mesothelial cells can grow and proliferate in commonly used tissue culture medium for 6-9 generations, and survive 1-2 months. In the present study, we did not use a medium containing epidermal growth factor, hydrocortisone, or iron-fortified calf serum,[19] because in this study, we wanted to study mesothelial cell functioning soon after the cells were taken from the human body, instead of using a long-term culture of mesothelial cells whose functioning might already have been changed by the agents added in the medium. For example, addition of epidermal growth factor to the culture medium could stimulate mesothelial cell growth.[20]

We found that IL-4, IL-7, and IL-10 were useless in the stimulation of the cellular functioning of mesothelial cells. There were also some minor differences among the mesothelial cells that were reactivated with IL-2 only, IL-2 plus IL-12, or IL-2 plus αCD3 stimulation. IL-2 plus IL-12- or αCD3-stimulated mesothelial cells had a higher IFNγ/IL-10 ratio than those stimulated with IL-2 alone, although not of statistical significance (Table 2). For cytolytic activity against autologous tumors, IL-2 plus αCD3-stimulated mesothelial cells had a higher, but insignificant, activity than IL-2-stimulated mesothelial cells. In contrast, cytolytic activity against K562 cells, indicating a non-specific killing activity, was also increased under these conditions.

Our previous studies revealed that lymphocytes isolated from malignant pleural effusion were in an immunosuppressed status that could be reversed with cytokine stimulation, and the restoration of functions required two-signal stimulation.[16] In contrast, IL-2 alone was enough to induce mesothelial cell proliferation and Th-1 pathway cytokine production, and to kill autologous tumor cells.

Activated lymphocytes, isolated from malignant pleural effusion after double-signal stimulation, may be an ideal source of effectors for use in adoptive immunotherapy.[16] They can be obtained in large quantities and can be activated to produce high levels of cytolytic activity against autologous tumors. The use of EAL or the direct manipulation of EAL in the pleural cavity may prove to be an alternative method to treat patients with malignant effusion.[16] However, it was more difficult to use activated mesothelial cells for adoptive immunotherapy because of the difficulty in obtaining large quantities of mesothelial cells, and because the activated cytolytic activity of the mesothelial cells was not higher than that of the activated lymphocytes from malignant pleural effusion, when compared with our previous studies.[14-16] Only a small minority of patients with malignant pleural effusion was able to yield enough mesothelial cells with/without cancer cells. The present study covered 2 years, with a collection of more than 200 cases of malignant pleural effusion, of which only 27 cases were successfully separated and yielded enough mesothelial cells for the tests (Fig. 3 and Table 4). In addition, there still may have been a small amount of lymphocytes that could not be completely separated from the mesothelial cells (including those lympho-

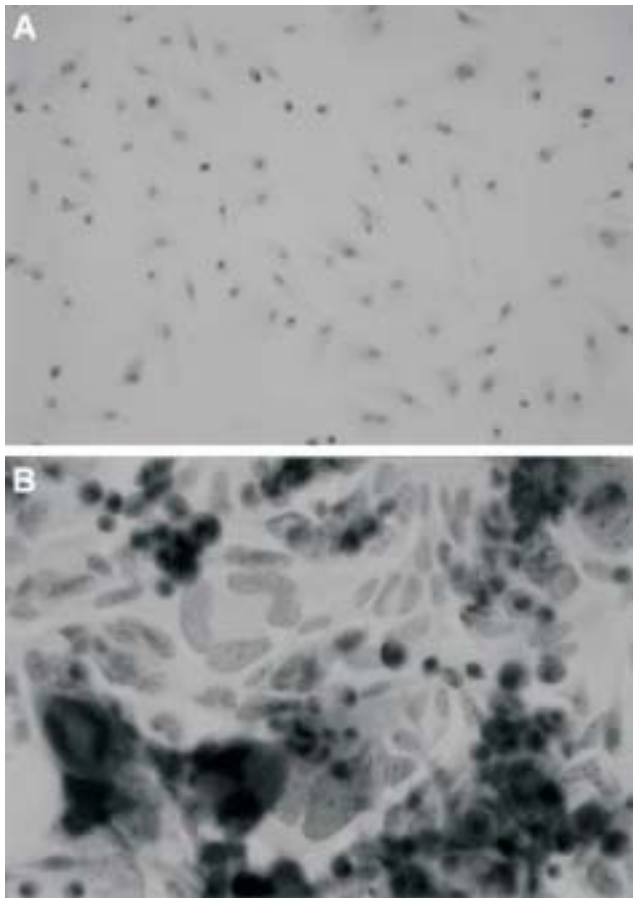

Fig. 3. Two photos of a representative primary culture of mesothelial cells and tumor cells, stained using the Papanicolaou method. *Panel A.* Mesothelial cells. *Panel B*. Tumor cells.

Table 4. Effusion characteristics and patient outcome (n = 27)

Case No.*	Effusion color/effusion amount/cell harvested (M/T)	P.I. after IL-2 treatment	IFNr/IL-10 ratio	Cytotoxicity at E/T of 15/1	Survival (months)
1	Straw, 800 mL, (22/1)	9.6	3.3	51	10
2	Straw, 900 mL, (66/0.2)	2.3	13.4	22	> 20
3	Yellow, 1000 mL, (60/3)	4.4	7.2	-	4
4	Straw, 800 mL, (30/3)	13.5	214.5	-	8
5	Yellow, 750 mL, (33/0.5)	3.8	3.4	-	> 18
6	Straw, 650 mL, (15/0.3)	4	7.6	-	14
7	Red, 900 mL, (57/0.1)	8.5	0	-	6
8	Pink, 880 mL, (31/2)	1	-	-	4
9	Brown, 850 mL, (15/3)	-	-	28.1	11
10	Straw, 450 mL, (4/1)	-	-	63.9	12
11	Yellow, 400 mL, (7/2)	1.2	-	26	4
12	Red, 800 mL, (13/1)	2.3	-	8	> 26
13	Yellow, 950 mL, (5/0)	1.8	-	-	8
14	Straw, 800 mL, (4/0)	2.2	-	-	9
15	Red, 500 mL, (2/0)	3	-	-	29
16	Pink, 700 mL, (1/0)	1.46	-	-	11
17	Straw, 600 mL, (1/4)	1.87	-	-	7
18	Red, 900 mL, (2/0)	22.41	-	-	14
19	Red, 850 mL, (2/0)	3.75	-	-	18
20	Straw, 400 mL, (3/0)	41.9	-	-	7
21	Yellow, 750 mL, (3/0)	2.38	-	-	9
22	Straw, 900 mL, (4/0.1)	7.1	-	-	14
23	Straw, 1100 mL, (55/4)	-	-	29	12
24	Yellow, 950 mL, (35/3.5)	-	-	3.52	10
25	Pink, 840 mL, (6/0)	1.7	-	-	6
26	Straw, 900 mL, (3/0.1)	2.4	-	-	9
27	Red, 1000 mL, (2/0.1)	5.1	-	-	25

M/T = harvested viable mesothelial cell/tumor cells, at 10^6/mL; P.I. = proliferation index.
*Cases 1-22 were pulmonary adenocarcinoma, cases 23-26 were poorly differentiated carcinoma of the lung, case 27 was breast adenocarcinoma.

cytes that could have been attached to the culture plate and/or mesothelial cells). Thus, all the immune responses mentioned in the present study could also have been affected by the remaining lymphocytes. Nevertheless, activated mesothelial cells might be used in conjunction with activated lymphocytes in order to get a higher immune response to cancer cells.

In summary, mesothelial cell proliferative and cytolytic activity can be enhanced with IL-2 stimulation alone. The T-helper pathway can also be shifted from Th-2 to Th-1 responses (increase of IFNγ/IL-10 ratio) after IL-2 stimulation of mesothelial cells. Gaining a more clear understanding of the effects of mesothelial cells on lymphocytes is deserving of further study.

REFERENCES

1. Dobbie JW. From philosopher to fish: the comparative anatomy of the peritoneal cavity as an excretory organ and its significance for peritoneal dialysis in man. *Peritoneal Dialysis Int* 1988;8:3.
2. Antony VB, Mohammed KA. Pathophysiology of pleural space infections. *Semin Respir Infect* 1999;14:9-17.
3. Li FK, Davenport A, Robson RL, Loetscher P, Rothlein R, Williams JD, *et al*. Leukocyte migration across human peritoneal mesothelial cells is dependent on directed chemokine secretion and ICAM-1 expression. *Kidney Int* 1998;54: 2170-83.
4. Mohammed KA, Nasreen N, Ward MJ, Mubarak KK, Rodriguez-Panadero F, Antony VB. Mycobacterium-mediated

chemokine expression in pleural mesothelial cells: role of C-C chemokines in tuberculous pleurisy. *J Infect Dis* 1998;178: 1450-6.

5. Zeillemaker AM, Mul FP, Hoynck, Leguit P, Verbrugh HA, Roos D. Neutrophil adherence to and migration across monolayers of human peritoneal mesothelial cells. The role of mesothelium in the influx of neutrophils during peritonitis. *J Lab Clin Med* 1996;127:279-86.
6. Antony VB, Hott JW, Kunkel SL, Godbey SW, Burdick MD, Strieter RM. Pleural mesothelial cell expression of C-C (monocyte chemotactic peptide) and C-X-C (interleukin 8) chemokines. *Am J Respir Cell Molecular Biol* 1995;12:581-8.
7. Zeillemaker AM, Mul FP, Hoynck van Papendrecht AA, Kuijpers TW, Roos D, Leguit P, *et al.* Polarized secretion of interleukin-8 by human mesothelial cells: a role in neutrophil migration. *Immunology* 1995;84:227-32.
8. Betjes MG, Tuk CW, Struijk DG, Krediet RT, Arisz L, Hart M, *et al*. Interleukin-8 production by human peritoneal mesothelial cells in response to tumor necrosis factor-alpha, interleukin-1, and medium conditioned by macrophages co-cultured with Staphylococcus epidermidis. *J Infect Dis* 1993;168:1202-10.
9. Goodman RB, Wood RG, Martin TR, Hanson-Painton O, Kinasewitz GT. Cytokine-stimulated human mesothelial cells produce chemotactic activity for neutrophils including NAP-1/IL-8. *J Immunol* 1992;148:457-65.
10. Gjersten MK, Saeterdal I, Beiske K, Gaudernack G. Antigen-presenting function of human peritoneum mesothelial cells isolated from a pancreatic carcinoma patient after mutant Ras peptide vaccination. *Cancer Immunol Immunother* 1996; 43:262-8.
11. Sahn SA. The pleura. *Am Rev Respir Dis* 1988;138:184-234.
12. Light RW. Pleural diseases. *Disease-a-Month* 1992;38:261-331.
13. Chen YM, Yang WK, Whang-Peng J, Kuo BI, Perng RP. Elevation of interleukin-10 levels in malignant pleural effusion. *Chest* 1996;110:433-6.
14. Chen YM, Yang WK, Ting CC, Tsai WY, Yang DM, Whang-Peng J, *et al*. Cross regulation by IL-10 and IL-2/IL-12 of the cytolytic activity of lymphocytes from malignant effusion of lung cancer patients. *Chest* 1997;112:960-6.
15. Chen YM, Yang WK, Whang-Peng J, Tsai WY, Hung YM, Yang DM, *et al*. Both IL-2 stimulation and TCR-CD3 engagement are required to reverse the *in vivo* anergized tumor-specific CTL from lung cancer patients. *J Immunotherapy* 1997; 20:354-64.
16. Chen YM, Tsai CM, Whang-Peng J, Perng RP. Double-signal stimulation was required for full recovery of effusion-associated lymphocytes' killing of autologous tumors. *Chest* 2002; 122:1421-7.
17. Kung PC, Goldstein G, Reinherz EL, Schlossman SF. Mono clonal antibodies defining distinctive human T cell surface antigens. *Science* 1979;206:347-9.
18. Lanfrancone L, Boraschi D, Ghiara P, Falini B, Grignani F, Peri G, *et al*. Human peritoneal mesothelial cells produce many cytokines (granulocyte colony-stimulating factor [CSF], granulocyte-monocyte-CSF, macrophage-CSF, interleukin-1 [IL-1], and IL-6) and are activated and stimulated to grow by IL-1. *Blood* 1992;80:2835-42.
19. Murphy JE, Rheinwald JG. Intraperitoneal injection of genetically modified, human mesothelial cells for systemic gene therapy. *Human Gene Therapy* 1997;8:1867-79.
20. van Rossen MEE, Hofland LJ, van den Tol MP, van Koetsveld PM, Jeekel J, Marquet RL, *et al.* Effect of inflammatory cytokines and growth factors on tumor cell adhesion to the peritoneum. *J Pathol* 2001;193:530-7.