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M. Loui Thomas · Urmila C. Samant
Ramakant Krishnaji Deshpande
Shubhada Vivek Chiplunkar

γδ T cells lyse autologous and allogenic oesophageal tumours: involvement of heat-shock proteins in the tumour cell lysis

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Abstract T cells expressing γδ receptors were isolated from the peripheral blood of oesophageal cancer patients and analysed for their potential to lyse tumour targets. Immunophenotyping by flow cytometry showed that the dominant population of γδ T cells expressed the Vγ9 and the Vδ2 T cell receptor, and a minor population expressed the Vδ1 receptor. Cytotoxicity assays revealed that activated γδ T cells lysed Daudi Burkitt's lymphoma and K562 cells. Lysis of autologous oesophageal tumours was higher than of allogenic tumours. Anti-hsp60 and anti-hsp70 mAb significantly inhibited the cytotoxicity of γδ T cells to both autologous and allogenic oesophageal tumours. Surface expression of hsp60 and hsp70 on oesophageal tumours and Daudi cells was demonstrated by flow cytometry. In conclusion, γδ T cells isolated from the peripheral blood of oesophageal cancer patients have the ability of kill oesophageal tumour cells. The lysis of tumour targets by the γδ T cells is brought about via recognition of heat-shock proteins expressed on the surface of tumour cells. γδ T cells isolated from the peripheral blood may have applications in adoptive immunotherapy of oesophageal cancer.

M.L. Thomas
Cellular Immunology Unit, Cancer Research Institute,
Tata Memorial Centre, Parel, Mumbai-400012, India

U.C. Samant
Tumour Marker Laboratory, Tata Memorial Hospital,
Tata Memorial Centre, Parel, Mumbai-400012, India

R.K. Deshpande
Department of Surgery, Tata Memorial Hospital,
Tata Memorial Centre, Parel, Mumbai-400012, India

S.V. Chiplunkar (✉)
Officer-in-Charge, Cellular Immunology Unit,
Cancer Research Institute,
Tata Memorial Centre, Parel, Mumbai-400012, India

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Introduction

T cells expressing γδ T cell receptors (TCR) are a minor population in the peripheral blood (< 10%) compared to the cells expressing αβ TCR (> 90%). Recent studies demonstrate that γδ T cells participate in immune responses in infectious, autoimmune and neoplastic diseases [3, 6, 27]. These lymphocytes have unique features with respect to their ontogeny, tissue tropism and receptor diversity. Subsets of γδ T cells appear to be strategically distributed throughout the epithelial layers of the body, in strong contrast to the apparent random localization of αβ T cells. γδ T cells have been found infiltrating breast, lung and renal tumours [7, 2, 1].

The prognosis for oesophageal carcinoma still ranks with those of the most aggressive cancers [9], and a profound regional immunosuppression has been reported in these patients [17]. Lymphokine-activated killer (LAK) cells are difficult to generate from these patients and this may be an impediment to developing potential immunotherapeutic strategies. It is therefore necessary to explore other subsets of lymphocytes that may have immunotherapeutic potential.

We report here the enrichment of γδ T cells from the peripheral blood of oesophageal cancer patients and an analysis of their immunophenotype and function. The studies demonstrate that γδ T cells have the ability to lyse autologous and allogenic oesophageal tumours. The cytotoxicity of γδ T cells against tumour cells is mediated via recognition of the heat-shock proteins hsp60 and hsp70 expressed on the surface of the tumour cells. Our recent studies have demonstrated that γδ T cells are able to recognize hsp60 expressed on oral tumour cells [15]. In the present study, the ability of γδ T cells to recognize both hsp60 and hsp70 expressed on oesophageal tumour cells has been investigated.

Materials and methods

Study group

Patients ($n = 10$) undergoing oesophageal resection for squamous carcinoma in the distal half of the oesophagous, with or with out lymph node involvement, were included in these studies. All the patients were staged according to the TNM system of classification and patients in all the four stages (stages I–IV) were included in the study. Blood samples were collected from the patients prior to surgery and the tumour tissues from the same patients (autologous tumours) were collected immediately after the surgery. Tumours collected from another set of oesophageal cancer patients (stages I–IV) served as the source of allogenic tumours.

Monoclonal antibodies

BMA031 (pan αβ T cell receptor) and Vδ1 (variable δ1 chain) were obtained from T Cell Sciences, USA. TCRδ1 (pan γδ TCR), 7A5 (Vγ9) and BB3 (Vδ2) were a gift from Dr. D. Kabelitz, Germany. Fluorescein isothiocyanate (FITC) conjugates and anti-hsp70 were from Sigma, USA. mAb IA-1, recognising an epitope located between amino acids 172 and 224 of the bacterial hsp65, was a gift from Dr. De Bruyn, Belgium.

Establishment of γδ T cells

The γδ T cells were enriched from the peripheral blood of oesophageal cancer patients as described earlier [15]. Peripheral blood mononuclear cells (PBMC, 3×10^5 cells) were cocultured with mitomycin-C-treated Daudi cells (2×10^5 cells) in 24-well tissue-culture plates (Nunc, Denmark) in RPM1-1640 medium (Gibco BRL, N.Y.) supplemented with 10% inactivated human AB blood group serum and antibodies (penicillin 100 IU/ml, streptomycin 100 μg/ml, mycostatin 5 μg/ml). On day 4, 30 U recombinant interleukin-2 (rIL-2)/ml (a gift from Hoffmann La Roche, Switzerland) was added to the cultures. Thereafter rIL-2-supplemented medium was added twice weekly. On day 20, the cells were harvested and γδ T cells were isolated by depleting the αβ T cells by an immunomagnetic bead separation method [15]. Briefly the T cells were treated with anti-(TCR αβ) mAb (BMA 031) for 45 min on ice, washed and incubated with magnetic beads coated with sheep anti-(mouse IgG) (Dynabeads M280, Dynal, Oslo, Norway) for 2 h at 4 °C on a rotating shaker. Rosetted cells were removed by adherence to a magnet. The γδ T cells obtained in the supernatant were more than 98% viable and were further immunophenotyped.

Isolation of tumour cells

Autologous and allogenic oesophageal tumour cells used in the cytotoxicity and flow-cytometry experiments were obtained as described by Shimizu et al. [21]. Briefly tumour tissues from oesophageal cancer patients were collected after surgery and the necrotic, haemorrhagic and fatty tissues were removed. The tumour tissues were minced finely and incubated in RPMI medium containing, double-strength antibiotics and enzyme mixture (0.05% collagenase, 0.02% DNase and 5 U/ml hyaluronidase along with 1 mg/ml trypsin; Sigma, USA) at 37 °C for 2 h with intermittent shaking. The tumour tissue was then passed through a 200-gauge wire mesh to obtain a single-cell suspension. The cells were washed with RPMI medium containing double-strength antibiotics. The tumour cells and the infiltrating lymphocytes were separated on a discontinuous ficoll-hypaque (Sigma, USA) density gradient (75%–100%). Tumour cells can be collected from the interface between the medium and 75% ficoll-hypaque. The cells were washed to remove the ficoll-hypaque and the cell viability was checked by staining the cells with erythrosin B dye. The tumour cells isolated by the above method were frozen in a freezing mixture (90% fetal calf serum and 10% dimethylsulfoxide) in liquid nitrogen for further use. Whenever needed the tumour cells were revived, tested for viability (more than 98%) and used directly in the assays.

Cytotoxicity assays

γδ T cell cytotoxicity against various tumour targets (Daudi, K562, Molt-4, autologous and allogenic oesophageal tumours) was assessed in a standard 4-h chromium-release assay (^{51}Cr; Amersham, Buckinghamshire, England). ^{51}Cr-labelled target cells (2×10^3 cells/well in triplicate) were incubated in U-bottom 96-well microtitre plates (Nunc, Roskilde, Denmark) with effector γδ T cells at an effector:target (E:T) ratio of 30:1. This E:T ratio was determined after prior titration of effector cells. A maximum cytotoxicity of target cells was obtained at an E:T ratio of 30:1. After 4 h of incubation, 100-μl aliquots of the supernatants were collected and counted on a gamma counter. The maximum and spontaneous release (cpm) of ^{51}Cr were obtained by incubating labelled targets with 10% Triton X-100 (Sigma, USA) and complete medium respectively. The percentage specific lysis of ^{51}Cr - labelled cells was calculated as [(experimental release − spontaneous release)/maximum release − spontaneous release)] × 100.

In a separate set of experiments, ^{51}Cr-labelled target cells (autologous or allogenic tumour cells) were incubated with anti-hsp60 mAb (IA-1) and anti-hsp70 mAb before being mixed with the effectors. In another set, ^{51}Cr-labelled target cells were mixed with γδ effector T cells preincubated with anti-TCR antibodies (pan γδ TCR, Vγ9, Vδ2, Vδ1, and pan αβ TCR). The antibodies were incubated with the target or the effector cells for 30–45 min at 37 °C at predetermined saturating concentrations. Untreated effectors mixed with ^{51}Cr-labelled targets served as controls for the inhibition experiments. Inhibition of lysis was calculated as

$$\text{Inhibition } (\%) = 1 - \frac{\text{cytotoxicity in the presence of mAb}}{\text{cytotoxicity in the absence of mAb}} \times 100$$

Immunophenotyping using single-colour flow cytometry

Samples comprising 5×10^5 γδ T cells were incubated with mAb against CD3, Vγ9, Vδ2, Vδ1, and pan αβ TCR and pan γδ TCR followed by FITC-conjugated rabbit anti-(mouse IgG) for 30 min at 4 °C. For the analysis of surface expression of hsp, tumour cells (Daudi and oesophageal tumour cells) were incubated with mAb IA-1 (anti-hsp60) and anti-hsp70 followed by FITC-conjugated rabbit anti-(mouse IgG). Single-colour flow cytometry was performed on a FACScalibur (Becton Dickinson) or a EPICS-753 (Coulter Hialeah, Fla.) flow cytometer with a 488-nm argon laser, and 10 000 events were analysed for each sample. Cells stained with mouse isotype (all subtypes) conjugated to FITC served as the negative control.

Results

Flow cytometric analysis of γδ T cells enriched from the peripheral blood

Peripheral blood mononuclear cells (PBMC) from oesophageal cancer patients were cultured with mitomycin C-treated Daudi cells and rIL-2 for 20 days. After immunomagnetic separation the lymphocytes were analysed for TCR gene usage by immunophenotyping using a set of monoclonal antibodies against the TCR γδ framework (TCRδ1) and the TCRαβ framework (BMA031), and antibodies against Vγ9, Vδ2 and Vδ1 specific for the γδ T cell subset.

Flow-cytometric analysis demonstrated that the majority of the lymphocytes were $CD3^+$ (95%–98%) and dominantly expressed the γδ TCR (96%–98%) and a very low percentage expressed αβ TCR (< 2%). Analysis with subset-specific mAb revealed that the majority of γδ T cells were of the Vγ9 (86%–88.9%) and Vδ2 (85%–88%) phenotype, while a few γδ T cells were Vδ1-positive (4.2%–9%). A representative histogram showing the flow-cytometric analysis of purified γδ T cells in given in Fig. 1. The cytotoxic potential of purified γδ T cells against autologous and allogenic tumour targets was analysed in cytotoxicity assays.

Cytotoxicity of γδ T cells against oesophageal tumours

The ability of γδ T cells enriched from the peripheral blood of oesophageal cancer patients to lyse autologous and allogenic oesophageal tumours and tumour cell lines Daudi, K562 and Molt-4 was analysed in a standard 4-h ^{51}Cr-release assay. In order to determine the optimal E:T ratio at which the targets would be lysed efficiently, the γδ T cells (effectors) were mixed with tumour cells (targets: Daudi, autologous and allogenic oesophageal tumour cells) at E:T ratios of 60:1, 30:1, 15:1, 7.5:1, 3.75:1 or 1.5:1. As seen in Fig. 2A, B γδ T cells enriched from two oesophageal cancer patients showed maximum cytotoxicity towards Daudi, autologous and allogenic oesophageal tumour cells at E:T ratios of 30:1 and 60:1. Therefore, in all the subsequent cytotoxicity experiments, an E:T ratio of 30:1 was used.

As seen in Table 1, the γδ T cells preferentially lysed Daudi (41 ± 4%), autologous oesophageal tumours (36 ± 6%) and K562 cells (28 ± 7%) whereas they exhibited low cytotoxicity against Molt-4 (14 ± 4%). A comparison between the ability of γδ T cells to lyse autologous and allogenic oesophageal tumours revealed that γδ T cells showed higher cytotoxicity against autologous tumours (mean 36 ± 6%, range 10%–68.3%) than against allogenic tumours (mean 15 ± 3%, range 3%–39.9%). No correlation was observed in the ability

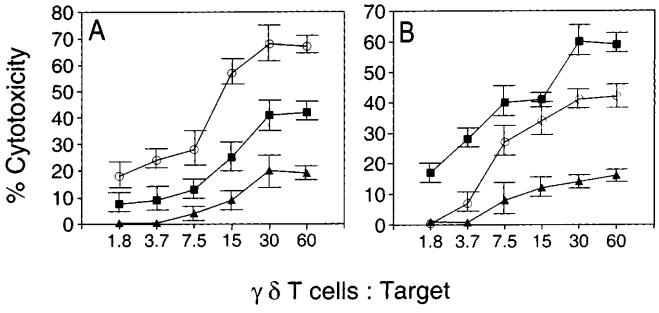

Fig. 2A, B Titration of cytotoxicity mediated by γδ T cells against Daudi (○), autologous (■) and allogenic (▲) oesophageal tumours. ^{51}Cr-labelled tumour targets were incubated with γδ T cells at various γδ-T-cells:target ratios in a standard ^{51}Cr-release assay. Data represent the mean ± SE of a cytotoxicity assay done on γδ T cells isolated from two patients (**A**, **B**)

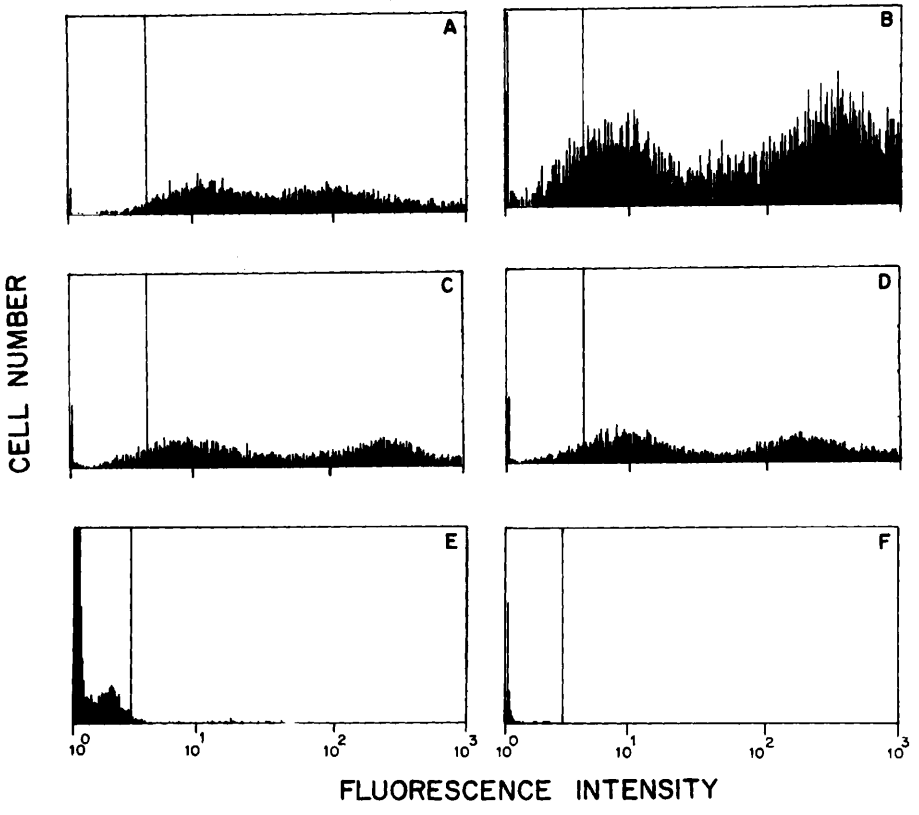

Fig. 1A–F Flow-cytometric analysis of γδ T cells isolated from the peripheral blood of oesophageal cancer patients. A representative histogram of γδ T cells stained with mAb to CD3 (**A**), TCRδ1 (**B**), Vγ9 (**C**), Vδ2 (**D**), Vδ1 (**E**) and BMA031 (**F**) is shown. The vertical line indicates the position of the isotype control and the histogram represents the percentage of cells positive for the mAb used. 10 000 events were analysed on an EPICS-753 flow cytometer

of γδ T cells to lyse autologous/allogenic oesophageal tumours and their TNM classification.

Expression of heat-shock proteins on tumour cells

The expression of heat-shock proteins (hsp60 and hsp70) on the surface of Daudi and oesophageal tumour cells was analysed by flow cytometry using mAb IA-1 (anti-hsp60) and anti-hsp70 mAb. Daudi cells showed a high surface expression of both hsp60 (mean 47 ± 8%, range 36%–58% $n = 8$) and hsp70 (mean 35 ± 7%, range 26%–44%, $n = 8$). In contrast the oesophageal tumours, both autologous and allogenic, showed a varying expression of hsp60 (mean 26 ± 9%, range 12%–40%, $n = 8$) and hsp70 (mean 30 ± 4%, range 20%–40%, $n = 8$) on the surface. As positive controls, the oesophageal tumours were simultaneously analysed for MHC class I expression, which ranged from 26% to 50%. Daudi cells do not express MHC class I and were therefore analysed for MHC class II expression, which ranged from 80% to 87%.

A representative histogram of Daudi and oesophageal tumour cells stained with mAb to hsp60 and hsp70 is shown in Fig. 3.

Inhibition of cytotoxicity mediated by γδ T cells by mAb

In order to demonstrate that the lysis of tumour targets by γδ T cells may be mediated via recognition of heat-shock proteins on their surface, inhibition of cytotoxicity by relevant mAb was carried out on γδ T cells isolated from four oesophageal cancer patients. γδ T cells were treated with mAb TCRδ1 (anti-TCR γδ), BMA031 (anti-TCRαβ) anti-Vγ9, anti-Vδ2 and anti-Vδ1 to identify the

Table 1 Cytotoxicity of γδ T cells against tumour targets was analysed in a ^{51}Cr-release assay at an E:T ratio of 30:1. Values in the table represent the percentage cytotoxicity ± SE. The mean cytotoxicity of γδ T cells against autologous oesophageal tumour cells was significantly higher ($P < 0.01$) than that against to allogenic oesophageal tumours. *ND* not done. The TNM classification of the tumours is shown in parentheses

Oesophageal cancer patient	Autologous oesophageal tumour	Allogenic oesophageal tumour	Daudi	K562	Molt-4
ESP1	18.7 ± 3 (T2N2M1)	39.9 ± 4 (T3N0M0)	20 ± 2	13.5 ± 2	2.1 ± 1
EPS2	68.3 ± 4 (T3N0M0)	3.7 ± 1 (T2N2M1)	40 ± 7	26 ± 4	ND
ESP3	48 ± 7 (T2N2M1)	20.7 ± 2 (T1N0M0)	30.2 ± 6	1.3 ± 1	8 ± 2
ESP4	41 ± 3 (T3N1M0)	8.8 ± 2 (T2N2M1)	42 ± 4	55 ± 4	17 ± 5
ESP5	10 ± 1 (T3N0M0)	3 ± 1 (T3N0M0)	40.9 ± 5	20 ± 4	18 ± 3
ESP6	11 ± 2 (T3N0M0)	17.4 ± 4 (T2N2M1)	43.1 ± 4	45.8 ± 5	9 ± 4
ESP7	28.6 ± 2 (T2N0M0)	21.5 ± 5 (T2N2M1)	52 ± 8	26 ± 3	11 ± 2
ESP8	30 ± 4 (T3N0M0)	13 ± 3 (T3N0M0)	60 ± 5	ND	ND
ESP9	60 ± 3 (T1N0M0)	11 ± 2 (T3N1M1)	19 ± 3	4.4 ± 2	9 ± 3
ESP10	39 ± 3 (T3N0M0)	9.2 ± 2 (T1N0M0)	56 ± 7	60 ± 6	40 ± 2
Mean ± SE	36 ± 6	15 ± 3	41 ± 4	28 ± 7	14 ± 4

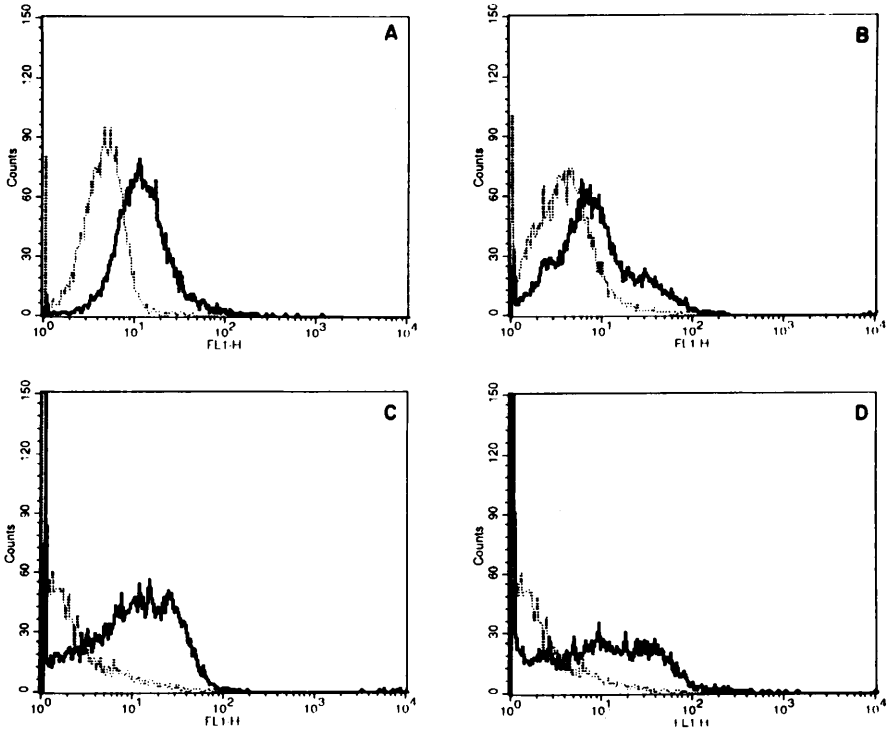

Fig. 3A–D Analysis of heat-shock protein (hsp60 and hsp70) expression on the surface of Daudi (*upper panels*) and oesophageal tumour cells (*lower panels*) by flow cytometry. **A, C** Expression of hsp60 on Daudi (58%) and oesophageal tumour cells (40%) respectively. **B, D** hsp70 expression on Daudi (26%) and oesophageal tumour cells (40%) respectively. (....) The isotype control, (—) the cells stained positive for the respective mAb. 10 000 events were analysed for each sample on a FACScalibur flow cytometer

subset of γδ T cells involved in recognition of tumour target. The oesophageal tumours were treated with anti-hsp60 mAb and anti-hsp70 mAb to confirm the involvement of heat-shock protein molecules as ligands for γδ T cells. As seen in Fig. 4, the pattern of inhibition of cytotoxicity of γδ T cells, revealed by mAb against framework γδ T cells and their subsets, was similar for autologous and allogenic tumour cells. The cytotoxicity against autologous tumours was dominantly inhibited by mAb TCRδ1 (92 ± 8%), Vγ9 (86 ± 5%) and Vδ2 (84 ± 2%). Anti-Vδ1 mAb also inhibited (54 ± 5%) the cytotoxicity of γδ T cells but this activity was less than that of anti-Vδ2 mAb. Pan αβ mAb showed no significant inhibition of the cytotoxicity mediated by γδ T cells.

Similarly the lysis of allogenic tumours by γδ T cells was inhibited by mAb TCRδ1 (62 ± 7%), Vγ9 (80 ± 8%), Vδ2 (77 ± 4%) and Vδ1 (43 ± 9%).

A significant inhibition of cytotoxicity mediated by γδ T cells was observed when autologous oesophageal tumours were pretreated with anti-hsp60 (79 ± 9%) and anti-hsp70 (68 ± 10%) mAb. Similarly anti-hsp60 (93 ± 7%) and anti-hsp70 (73 ± 12%) mAb also showed increased inhibition of γδ T cell cytotoxicity towards allogenic oesophageal tumours.

Immunophenotypic analysis of γδ T cell subsets from these four oesophageal cancer patients revealed that the majority of the γδ T cells were of the Vγ9 and Vδ2 phenotype. A very low number of γδ T cells were positive for Vδ1 TCR (Table 2).

Table 2 Immunophenotype of γδ T cells. γδ T cells were stained with mAb to CD3, Vγ9, Vδ2, and Vδ1 followed by fluorescein-isothiocyanate-labelled anti-(mouse IgG). The cells were analysed on an EPICS 753 flow cytometer. Values in the table represent the percentage of positive cells for each of the mAb used

Oesophageal cancer patient	Cells (%) positive for:			
	CD3	Vγ9	Vδ2	Vδ1
ESP1	95	86	86	9
ESP3	98	88.9	89	4.2
ESP8	96.3	85.4	85	6.4
ESP9	95	ND	88	8

The results demonstrate that γδ T cells subsets expressing Vγ9, Vδ2 and Vδ1 TCR may be involved in the recognition of hsp60 and hsp70 molecules expressed on oesophageal tumours.

Discussion

The prognosis of patients with oesophageal cancer has not significantly improved during recent years. Even in resectable stages the results of standard therapy modalities (surgery or radiotherapy) have been poor, with 5-year survival rates of about 20% [16].

Adoptive immunotherapy of cancer is an attempt to use immunological manipulation of autologous lymphocytes to control tumour growth. To date, most studies have involved the use of LAK cells activated ex vivo by IL-2, or have involved tumor-infiltrating lymphocytes expanded ex vivo by IL-2 [19]. A failure to generate significant LAK responses in oesophageal cancer patients has motivated studies to investigate alternative strategies for immunotherapy. γδ T cells are known to exhibit potent cytotoxicity against tumour targets and have been implicated in adoptive immunotherapy of cancer [11].

In the present investigations we have enriched γδ T cells from peripheral blood of oesophageal cancer patients by incubating the PBMC with Daudi lymphoma cells known to expand γδ T cells selectively. We [15] and others [8, 22] have demonstrated earlier that stimulation of PBMC with Daudi cells, but not with any other B-lymphoblastoid cell line, results in the expansion of γδ T cells expressing Vγ9 and Vδ2 phenotypes. Coculturing of PBMC of oesophageal cancer patients also led to enrichment of the Vγ9 and Vδ2 phenotype, indicating a strong bias towards the use of Vγ9 and Vδ2 genes from the available gene repertoire of γδ T cells. Studies have suggested that the postnatal peripheral blood expansions of Vγ9/Vδ2 may be the result of exposure of the immune system to certain bacterial superantigens [24]. A small fraction of γδ T cells enriched from the peripheral blood of oesophageal cancer patients also showed the Vδ1

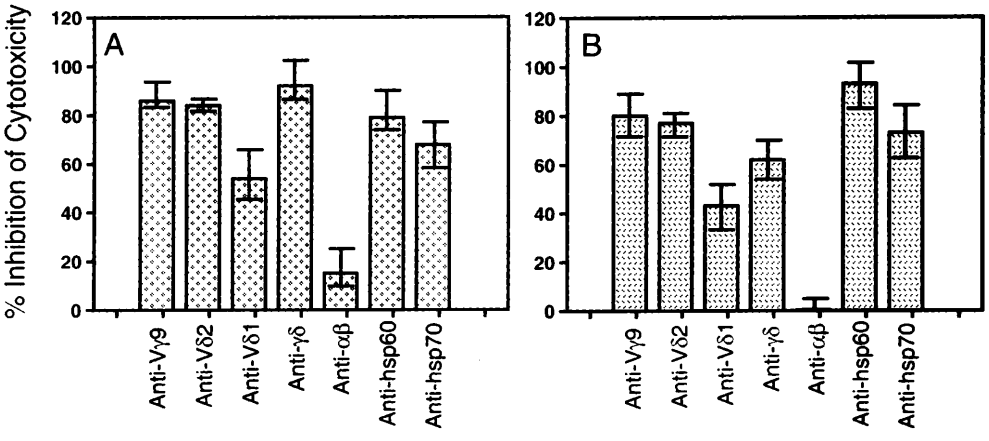

Fig. 4A, B Inhibition of γδ T cell cytotoxicity against autologous oesophageal tumours (**A**) and allogenic oesophageal tumours (**B**) by mAb. The cytotoxic potential of γδ T cells was assayed in the presence of mAb against TCRγδ, Vγ9, Vδ2, Vδ1, and TCRαβ. The tumour targets were pretreated with mAb to hsp60 and hsp70 and then incubated with the γδ T cells. Data represent the mean percentage inhibition of cytotoxicity ± SE against oesophageal cancer cells from four patients

phenotype. It has been demonstrated that, although in the peripheral blood Vγ9 dominantly pairs with Vδ2, a small population of the γδ T cells also show the Vγ9/Vδ1 pairing [20].

The cytotoxic potential of γδ T cells was analysed against Daudi, K562, Molt-4 cell lines and against a panel of autologous and allogenic oesophageal tumours. The γδ T cells showed higher cytotoxicity against Daudi cells than against K562, and a low cytotoxicity against Molt-4. γδ T cells are known to lyse both Daudi and K562 cells [25].

The cytotoxicity exhibited by γδ T cells against autologous oesophageal tumours was markedly higher than that against allogenic oesophageal tumours. A preferential lysis of autologous tumours by γδ T cells has also been reported in renal tumours and in dysgerminoma and seminoma. It appears unlikely that MHC restriction is involved in the preferential lysis of autologous tumours by γδ T cells, as none of the HLA- or CD1-specific mAb reduced the lytic activity of γδ T cell clones [2, 28]. The nature of the antigens recognized by γδ T cells is not fully understood. Heat-shock proteins and phosphoantigens are known to stimulate γδ T cells oligoclonally [12]. Using surface biotinylation and immunoprecipitations with mAb IA-1, we [15] and others [13] have demonstrated that Daudi cells express heat-shock protein 60 on their surface, which serves as a ligand for γδ T cells. The hsp60 expression is not observed on K562 cells [15].

Heat-shock proteins are a highly conserved group of intracellular proteins and their expression is increased in response to stress, such as infection and neoplasia [5]. The family of heat-shock proteins (hsp90, hsp70 and hsp60) expressed on the surface of malignant cell types are immunogenic tumour antigens and can evoke a cytotoxic T cell response [10]. Human hsp70 and bacterial hsp65 have significant sequence homology (30% similarity at gapweight 10) and hsp70 has 66% similarity at the gapweight 10 with the human homologue of the bacterial hsp65 [4]. In the present investigations we also analysed whether the oesophageal tumours express heat-shock proteins on the surface of cells and whether they serve as ligands for γδ T cells. Flow cytometry using the relevant mAb revealed expression of both hsp60 and hsp70 on the oesophageal tumours that ranged from 12% to 40% and from 20% to 40% respectively. Evidence has accumulated that hsp60, hsp70 and hsp90 are expressed on tumours of the uterine cervix and oral cavity [14, 18, 23]. The high constitutive expression of hsp60 that we observed on Daudi cells was also reported by other investigators [13, 15]. In addition the present studies also showed that hsp70 is expressed on Daudi cells.

In order to investigate whether γδ TCR is involved in the lysis of autologous and allogenic oesophageal tumours and whether heat-shock proteins expressed on tumours serve as ligands for γδ T cells, cytotoxicity experiments were conducted in the presence of the relevant mAb. Our results demonstrate that mAb against the TCR γδ framework (TCRδ1) and the Vγ9, Vδ2 and Vδ1 subsets of γδ T cells significantly inhibited the lysis of both autologous and allogenic oesophageal tumours. The Vγ9 and Vδ2 mAb inhibited the cytotoxicity of γδ T cells to oesophageal tumours to a greater extent than Vδ1 mAb. Although we have no direct evidence, our studies suggest that pairing of Vγ9 with Vδ2 or Vγ9 with Vδ1 may be occurring in the peripheral blood of oesophageal cancer patients. Subsets of γδ T cells expressing both phenotypes (Vγ9/Vδ2, Vγ9/Vδ1) are capable of lysing oesophageal tumours. Schondelmaier et al. [20] have demonstrated that, although Vδ1 dominantly pairs with Vγ2, 3, 4, a minor population of γδ T cells exist in the peripheral blood that show a Vγ9 and Vδ1 pairing. Our studies demonstrate that, although the Vδ1 subset forms a minor population of γδ T cells in the peripheral blood of oesophageal cancer patients, they exhibit strong tumour-directed cytolytic ability.

Treatment of autologous and allogenic oesophageal tumours with anti-hsp60 and anti-hsp70 mAb inhibited the lysis of these tumours by γδ T cells, indicating that γδ T cells mediate the lysis of these tumours through recognition of hsp60 and hsp70. In our studies we observed no correlation between the expression of hsp60/hsp70 on the autologous tumour cells and their preferential lysis by the γδ T cells. Zhao et al. [28] demonstrated that increased expression of hsp70 on seminoma and dysgerminoma cells make them susceptible to lysis by γδ T cells. Similarly, it was observed that reactivity of γδ T cells to autologous heat-stressed tumour cells was inhibited by anti-hsp70 mAb [26]. Recent studies from our laboratory [15] showed that γδ T cells from peripheral blood of oral cancer patients mediate lysis of oral tumours via recognition of hsp60 expressed on these tumours.

In conclusion, the present studies add a new dimension to the role of heat-shock proteins expressed on oesophageal tumours as antigens for γδ T cells. Moreover, these studies encourage further investigation of the role of γδ T cells in adoptive immunotherapy of oesophageal cancer.

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