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Modulatory Effect of Cytokines on Natural Killer and Antibody Dependent Cellular Cytotoxicity Directed to Squamous Cell Carcinoma Targets by Lymphocytes from Oral Cancer Patients

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Cells from solid tumours are generally poor targets for natural killer (NK) cytotoxicity and antibody dependent cellular cytotoxicity (ADCC). In this paper, we have analysed NK cytotoxicity and ADCC mediated by peripheral blood mononuclear cells from healthy individuals and oral cancer patients before and after modulation with recombinant interleukin-2 (rIL-2), on target cells derived from two squamous cell carcinoma (SCC) cell lines prior to and after treatment with recombinant interferon-α (rIFNα). Target SCC cell directed monoclonal antibody 3F8E3 was used in ADCC. The results showed that the unmodulated SCC cells were poor targets for NK and ADCC compared to standard targets (K562 cells and chicken red blood cells, respectively). Modulation of targets alone with rIFNα showed moderate increase in their susceptibility while rIL-2 treated effectors could significantly lyse even unmodulated targets. Combined treatment of targets with rIFNα and effectors with rIL-2 showed additive enhancement in NK and ADCC activity against SCC cells. Lymphocytes from treated patients with recurrent disease could not efficiently lyse SCC targets even after combined modulation.
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INTRODUCTION

A NUMBER of reports published in the past suggests the role of natural killer (NK) cells and antibody dependent cellular cytotoxicity (ADCC) in effective killing of targets *in vitro*, and also in immune surveillance *in vivo* [1, 2]. In most of the *in vitro* studies reported, susceptible targets have been used to measure the cytotoxic potential of effector cells. Recently, the importance of the ability of cytotoxic cells to kill tumour targets relevant to the host has been brought out [3]. Although, solid tumour cells lines have been used as targets in NK cytotoxicity and ADCC, they were not necessarily similar in histology to the tumour cells of the patients whose lymphocytotoxicity was being assessed [4]. In ADCC, several other factors like the antibody isotype, its binding affinity and expression of antigens on the target cells influence the effective lysis of tumour cells [5]. Antibodies of the isotype IgG2a and IgG3 have been found to be efficient in mediating ADCC by various effectors [5].

In vitro NK cell function can be modulated by incubating the effector cells with all species of interferon (IFN), and interleukin-2 (IL-2) [6]. IFN is also known to modulate cell surface expression of antigens on targets [7]. Based on these findings, treatment protocols involving the use of rIFN and rIL-2 in patients with solid tumour have been devised in an attempt to augment NK cell activity [8]. However, there are very few reports on the susceptibility of IFN treatment of human tumour cells to ADCC by antibodies directed to tumour associated antigens (TAA) [9].

We have reported earlier, establishment and characterisation of four squamous cell carcinoma (SCC) cell lines derived from human oral cancer tissues [10]. We have reported a monoclonal antibody (Mab) 3F8E3, of IgG3 isotype, which reacts with cultured SCC cells and SCC cells in tissue sections [11]. Further, we have shown that treatment of SCC cell lines with rIFNα increases the affinity of binding of Mab 3F8E3 to TAA expressed on the cells and inhibits its shedding [12]. In this paper we report NK cell mediated cytotoxicity and Mab 3F8E3 mediated ADCC of lymphocytes from oral cancer patients towards SCC cell lines after appropriate modulation of effectors with rIL-2 and targets with rIFNα.

MATERIALS AND METHODS

Lymphocyte donors

Peripheral blood was collected from 10 healthy donors and 10 oral cancer patients from three groups, comprising of untreated patients, treated patients with no evidence of disease (NED), and treated patients with recurrence (REC) of disease. Treated patients had undergone either surgery alone, or surgery with preoperative chemotherapy or radiotherapy, at least 6 months prior to the collection of the peripheral

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blood. They were in the age group 27–80 years with primary tumour sites involving lateral border of the tongue, gingiva of buccal sulcus, buccal mucosa, hard palate or retromolar trigone. Most of the patients had well to moderately differentiated SCC and were in stage III or IV of the disease. Few patients were in stage II, while none of the patients investigated were in stage I of the disease. Healthy individuals (age group 25–50 years) were mostly laboratory personnel, with no past history of any disease.

Separation of lymphocytes

Non-adherent peripheral blood mononuclear cells (PBMC) were used as effectors in the cytotoxicity assay.

Treatment of effectors and targets with cytokines

SCC cells (1×10^5) of cell lines AW 8507 and AW 13516 were seeded in culture flasks (NUNC, Denmark) and treated with 1000 U/ml of rIFNα (Ernest, Boehringer, U.S.A.) for 96 h. Untreated and rIFNα treated cells were used as targets for the cytotoxicity assay. K562 (an erythroleukaemic cell line) and chicken red blood cell (cRBC) coated with rabbit anti cRBC antibody (1:400) were used as standard targets for NK and ADCC, respectively. PBMC obtained from healthy individuals and oral cancer patients with or without incubation with 50 U/ml of rIL-2 (Cetus Corp., U.S.A.) for 18 h were used as effectors.

Antibodies

Mab 3F8E3 developed against a head and neck cancer cell line, HN2 [11] was purified from ascitic fluid using protein A sepharose (Pharmacia, Sweden) column chromatography. It was used in ADCC at a concentration of 1 μg/ml.

Cytotoxicity assay

A standard 4 h ^{51}Chromium (^{51}Cr as sodium chromate, BARC, Bombay, India, specific activity 555 MBq/mg) release assay was used to assess cytotoxicity at effector to target ratio of 50:1 [3].

RESULTS

NK and ADCC of unmodulated cells

Both NK and K cells (mediating ADCC) showed substantial lysis of standard targets, the cytotoxic potential being comparable in healthy individuals and oral cancer patients from all the three groups (untreated, NED and REC, Fig. 1). However, both the cytotoxic mechanisms showed very low potential to kill cultured SCC cells of cell lines AW 8507 and AW 13516.

Treatment of targets with rIFNα

Treatment of SCC cell lines with rIFNα increased their susceptibility to lysis by both NK and K cells from healthy individuals and treated oral cancer patients with no evidence of the disease (Table 1). However, the increase in per cent NK cytotoxicity after rIFNα modulation was not statistically significant. The increase in both NK and ADCC activities was still lower than that observed against standard targets (Table 1).

It is interesting to note that rIFNα modulated target cells failed to show augmented lysis by lymphocytes from treated oral cancer patients with recurrent disease (Table 1).

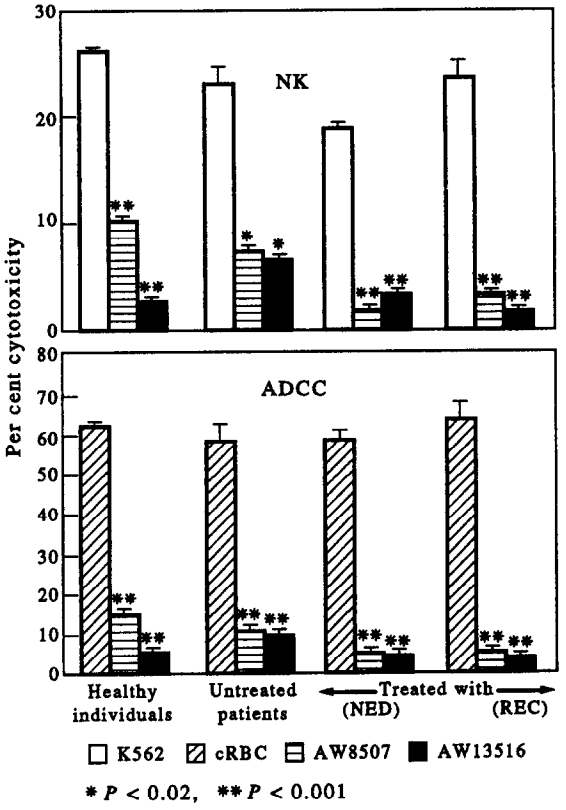

Fig. 1. NK and ADCC responses of unmodulated lymphocytes from healthy donors and oral cancer patients. Targets for NK: K562 and SCC cell lines AW 8507 and AW 13516. Targets for ADCC: cRBC and SCC cell lines AW 8507 and AW 13516.

Treatment of effectors with rIL-2

On the other hand, modulation of effectors from healthy individuals and oral cancer patients with rIL-2 showed significant increase in NK and ADCC activity against SCC cell lines compared to unmodulated targets (Table 1). It was observed that SCC cell line AW 8507 was more susceptible to lysis than AW 13516.

Modulation of effector cells from treated oral cancer patients (NED and REC) with rIL-2, although showed a significant increase in NK and ADCC activity, the resulting per cent cytotoxicity was much less (Table 1).

Treatment of targets with rIFNα and effectors with rIL-2

Combined treatment of SCC cell lines with rIFN α and effectors with rIL-2 showed additive modulatory effect (Table 1, Figs 2 and 3), for both the cell lines in NK cytotoxicity and ADCC assays. Even in the experiments dealing with combined modulation of targets and effectors, cell line AW 8507 showed better susceptibility to lysis than AW 13516. It could be noted that after modulation of targets and effectors with cytokines, in treated patients, only NK mediated lysis of SCC cells was comparable to standard targets (Fig. 2).

DISCUSSION

Earlier studies have established the existence of immunological defects in patients with head and neck cancer [13]. Decrease in T lymphocyte mediated immune responses were shown to be more pronounced in head and neck SCC patients [14]. Some studies have shown that head and neck cancer patients have deficient NK function [15], while our studies

Table 1. Modulation of NK cytotoxicity and ADCC

Source of lymphocytes and cytotoxic mechanisms	Targets	Cytokine treatment of lymphocytes (E) and targets (T) with: Nil	IFNα (T)	IL-2 (E)	IFNα (T) + IL-2 (E)
Healthy donors					
NK	AW 8507	10.2 ± 2.0	15.8 ± 2.3	37.1 ± 3.8§	43.5 ± 3.7§
ADCC		14.4 ± 2.3	21.6 ± 2.7	44.7 ± 4.0§	50.8 ± 4.0§
Untreated patients					
NK		6.8 ± 2.0	8.0 ± 2.7	33.6 ± 5.2§	41.9 ± 5.6§
ADCC		9.6 ± 2.5	12.6 ± 3.0	40.7 ± 5.2§	51.8 ± 6.5§
Treated patients (NED)					
NK		1.8 ± 0.5	5.2 ± 1.2†	11.6 ± 2.2‡	15.1 ± 3.6‡
ADCC		3.5 ± 0.7	6.1 ± 1.2‡	18.0 ± 2.6§	26.8 ± 2.8§
Treated patients (REC)					
NK		2.4 ± 0.7	2.2 ± 0.6	13.1 ± 3.5‡	13.5 ± 5.0*
ADCC		3.1 ± 0.8	3.3 ± 0.3	13.2 ± 3.4†	15.6 ± 4.7†
Healthy donors					
NK	AW 13516	2.5 ± 0.6	3.8 ± 0.8	12.8 ± 2.5§	14.4 ± 3.8‡
ADCC		4.9 ± 0.5	8.2 ± 0.6	20.2 ± 2.4§	21.3 ± 4.2‡
Untreated patients					
NK		6.0 ± 2.3	8.8 ± 3.5	19.1 ± 4.8*	20.4 ± 4.2‡
ADCC		8.8 ± 3.2	11.9 ± 3.8	23.9 ± 5.0*	27.1 ± 4.7‡
Treated patients (NED)					
NK		2.7 ± 0.9	9.8 ± 3.4	12.8 ± 4.3*	9.9 ± 2.7*
ADCC		3.4 ± 0.9	10.8 ± 3.5	13.5 ± 3.6†	10.6 ± 2.4†
Treated patients (REC)					
NK		0.9 ± 0.4	1.8 ± 0.6	6.4 ± 1.9†	10.6 ± 5.0
ADCC		1.6 ± 0.5	2.2 ± 0.7	7.2 ± 2.0†	13.3 ± 5.0*

P values when compared with unmodulated targets and effectors in respective groups.
*<0.05, †<0.02, ‡<0.01, §<0.001.

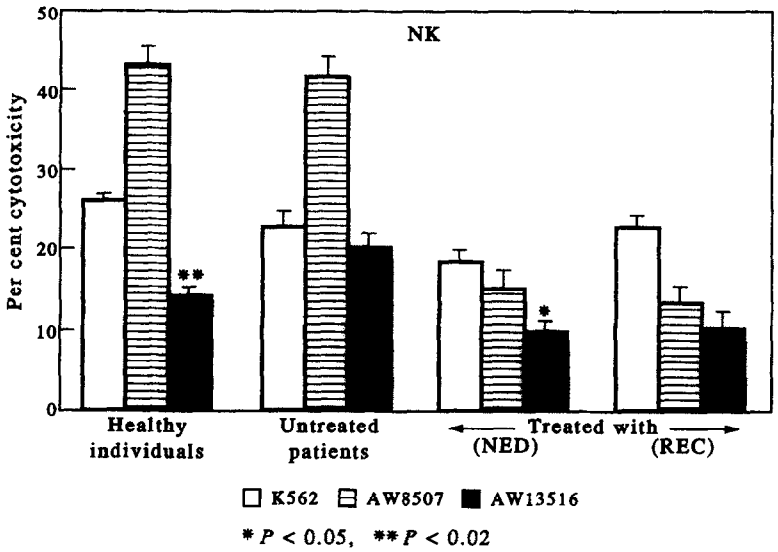

Fig. 2. Comparison of NK cytotoxicity of effectors from healthy donors and oral cancer patients on susceptible K562 targets and SCC targets. For NK cytotoxicity on SCC targets, effectors and targets were modulated with rIL-2 and rIFNα, respectively.

have shown that PBMC from oral cancer patients have normal NK and ADCC activity on susceptible targets. However, the NK and ADCC functions of regional lymph nodes and tumour infiltrating lyphocytes were compromised to a great extent [16]. The NK cell activity in patients in with head and neck cancer has been correlated with the pattern of recurrence [17]. In most of the studies mentioned above, the cytotoxity was assessed on susceptible targets. For the cytotoxic mechanism to be correlated with the tumour progression, the cytotoxic cells should have an ability to destroy relevant targets. It is with this viewpoint that we have undertaken studies to investigate the ability of NK and K cells from oral cancer patients to kill SCC cell lines—AW 8507 and AW 13516—derived from oral cancer tissues. The cytotoxicity was modified by cytokines rIFNα and rIL-2. It was observed that although NK and ADCC activity of effectors from healthy individuals and oral cancer patients against K562 and cRBC targets was comparable, when SCC cell lines were used as targets, the cytotoxicity was very low, thus indicating the need to modulate the effectors and targets suitably.

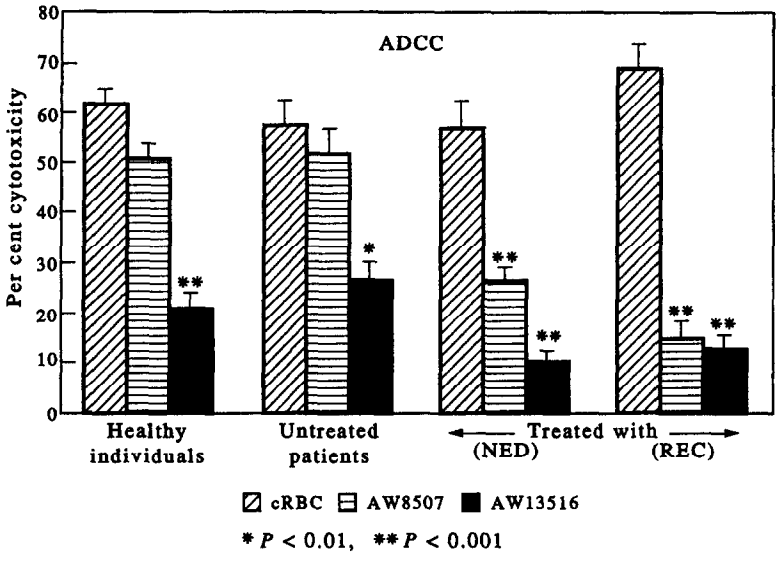

Fig. 3. Comparison of ADCC of effectors from healthy donors and oral cancer patients on susceptible cRBC targets and SCC targets. For ADCC on SCC targets, effectors and targets were modulated with rIL-2 and rIFNα, respectively.

IFN and IL-2 are known to potentiate cytotoxic function of NK cells [6]. Data are also available on the effect of IFN treatment at the level of target cell population. These include enhanced expression of major histocompatibility complex (MHC) Class I antigens, perhaps leading to a better susceptibility of targets to cytotoxic T-lymphocyte (CTL) mediated lysis [18], enhanced expressed of adhesion molecules such as ICAM-1, leading to better conjugation of targets and effectors [19] and modulation of TAA expression [8, 9, 12].

There are controversial views regarding the relationship between expression of class I antigen on targets and increased resistance to NK cell lysis [20, 21]. Cells of both the cell lines used targets in our studies expressed MHC class I antigens and were initially refractile to NK lysis [10]. It is possible that the susceptibility of rIFNα treated target cells to NK cell mediated lysis is a multifactorial event [22] and that factors like state of differentiation of target cells, alterations in glycosylation or the level of expression of adhesion molecules may significantly influence the susceptibility of targets to NK cell lysis [21]. While, for other cytotoxic mechanisms such as those mediated by K cells (ADCC) and CTL, expression of critical number of TAA on target cells could be an essential feature.

There has been a controversy with respect to the modulatory effect of IFN on the expression of TAA on tumour cells [7, 9]. We reported earlier that treatment of cultured SCC cells with rIFNα failed to show increased expression of TAA on SCC cells, but the binding affinity of the antibody was increased [12]. In the cytotoxicity assays presented here, although there was a trend towards increase in NK and antibody mediated cellular killing after treating the targets with IFN α, the difference was not significant. Moderate increase in cytotoxicity could be a result of better conjugation of effectors and targets via either increased expression of adhesion molecules as suggested by Naganuma *et al.* [19] or via increased affinity of Mab 3F8E3 [12].

Several reports have shown increased potential of effectors treated with rIL-2 to kill targets [6]. We have also demonstrated earlier that modulation of NK and K cells by IL-2 enhanced their lytic potential towards fresh leukaemic targets [23]. In the presented studies, substantial increase was seen in the cytotoxic potential of rIL-2 treated effectors to lyse SCC cell lines. Differential sensitivity of target cells obtained from two cell lines was quite evident.

Combined treatment of effectors with rIL-2 and oral targets with rIFNα further augmented the cytotoxicity in an additive fashion especially with the targets of AW 8507. Similar results have been reported by Naganuma *et al.* [19].

The NK cell mediated cytotoxicity and ADCC of lymphocytes from untreated oral cancer patients were comparable to those of healthy donors on susceptible as well as SCC targets. However, the treated patients, irrespective of their recurrence status, displayed compromised cytotoxic potential when SCC cells were used as targets. Modulation of effectors and targets with cytokines did not improve the cytotoxic potential of treated patients on SCC targets. This observation might indicate the prolonged inhibitory effect of treatment on immune cell functions in oral cancer patients.

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