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Comparison of the relative cytotoxic effector cell capabilities and the proportions of cells bearing various surface markers in human tonsil and peripheral blood mononuclear cells

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SUMMARY

The relative cytotoxic effector cell capabilities and the proportions of cells bearing various surface markers in human tonsil and peripheral blood mononuclear cells has been studied. The peripheral blood contained a substantial proportion of monocytes ($22 \pm 2{\cdot}9\%$) compared to tonsil cell suspensions ($2{\cdot}5 \pm 0{\cdot}3\%$). The percentages of T lymphocytes was significantly higher in the blood than in the tonsil ($P < 0{\cdot}01$); however, the percentages of cells forming rosettes with 7S EA were not significantly different in each group ($P > 0{\cdot}5$). Mitogen-induced cellular cytotoxicity by blood and tonsil mononuclear cells against Chang cells was proportional to the percentages of T lymphocytes in these cell suspensions, and both antibody-dependent and mitogen-induced cellular cytoxicity against sheep red blood cells was proportional to the percentages of monocytes in these suspensions. Tonsil mononuclear cell suspensions were incapable of mediating antibody-dependent cellular cytotoxicity against Chang cells, whereas blood mononuclear cells functioned normally. These findings are in contrast to the findings of similar percentages of Fc receptor-positive lymphocytes in blood and tonsil mononuclear cell suspensions. Previous studies have shown that the effector cells against antibody-coated Chang cells are Fc receptor-positive lymphocytes. These studies show that in the case of cytotoxicity mediated by an Fc receptor-bearing lymphoid cell, there may be a clear discrepancy between the relative proportions of Fc-bearing lymphoid cells in different organs and the relative levels of cytotoxicity.

INTRODUCTION

Non-immune mononuclear cells are capable of functioning in antibody-dependent (ADCC) and mitogen-induced (MICC) cellular cytotoxicity assays against various nucleated and non-nucleated target cells (Nelson *et al.*, 1976b, Perlmann & Holm, 1969). The identity of the subpopulations of effector cells which are capable of killing various target cells has recently been the subject of great interest. Previous studies of human mononuclear cells have demonstrated that both ADCC and MICC against non-nucleated erythrocytes are mediated by monocytes (Holm & Hammarström, 1973), whereas cells mediating either ADCC or MICC against Chang liver cell targets were non-adherent, non-phagocytic lymphoid cells (MacDonald *et al.*, 1975). MICC against Chang liver cells was mediated solely by thymus-derived lymphocytes (T-cells) and ADCC against these targets was mediated by surface Ig (sIg) negative, E-rosette negative, Fc receptor-bearing lymphocytes (Nelson *et al.*, 1976b). Nucleated erythrocytes are killed by multiple populations of effector cells (Hunninghake & Fauci, 1976b, c; Nelson *et al.*, 1976b). It has been previously noted that lymphocyte suspensions isolated from the lungs of guinea-pigs mediate MICC but not ADCC against Chang liver cell targets, even though a substantial

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proportion of these cells bear Fc receptors for 7S Ig (Hunninghake & Fauci, 1976a). In addition, O'Toole, Saxon & Bohrer (1977) have recently reported that human lymph node and thymus cells functioned poorly in ADCC. In humans, Holm *et al.*, (1974) demonstrated that thoracic duct lymphocytes could mediate MICC but not ADCC. These findings suggested that Fc receptor-bearing lymphoid cells from different organs possess varying cytotoxic capabilities in ADCC.

The present study was undertaken to determine the relationship between the relative cytotoxic effector capabilities of lymphoid cells in human tonsil and peripheral blood and the relative proportions of various populations of effector cells in these organs.

MATERIALS AND METHODS

Cell suspensions. Tonsils were obtained from subjects undergoing routine tonsillectomy for chronic tonsillitis. Mononuclear cells were obtained from teased tonsillar tissue as previously described (Fauci & Pratt, 1976) and further purified using standard Hypaque–Ficoll density centrifugation (Böyum, 1968). Peripheral blood was collected from healthy adults into heparinized syringes and mononuclear cells were obtained following Hypaque–Ficoll separation. The final mononuclear cell suspensions from both blood and tonsils were greater than 95% viable as measured by the trypan blue dye exclusion test.

Mononuclear cell subpopulations. The proportion of human lymphocytes forming rosettes with sheep red blood cells (SRBC) was used as an assay for T lymphocytes (Jondal, Holm & Wigzell, 1972).

Complement-binding mononuclear cells were identified by their ability to bind to 19S antibody and complement-coated SRBC (Bianco, Patrick & Nussenzweig, 1970). The number of mononuclear cells bearing receptors for immunoglobulin (Fc receptor) was determined by the ability of the cells to bind SRBC coated with rabbit IgG anti-SRBC antibody (Kedar, Oritz de Landazuri & Fahey, 1974). In some instances, where indicated, ox cells coated with rabbit IgG anti-ox cell antibody were used to determine the number of cells bearing receptors for 7S immunoglobulin and were prepared as described above. The assays noted above and used to determine the various mononuclear cell subpopulations were performed as previously described (Hunninghake & Fauci, 1976b; Fauci, 1975). Monocytes were identified by morphology and non-specific esterase stains (Jondal *et al.*, 1972).

Cytotoxicity Assay. ADCC and MICC were assayed by a modification of a previously described microcytotoxicity assay (Zeijlemaker *et al.*, 1975). Both assays employed the measurement of the release of isotyope from radioactive chromium ^{51}Cr-labelled SRBC or Chang cell target S (10^5 per assay) and were performed as previously described (Hunninghake & Fauci, 1976b). For the ADCC assay, rabbit anti-SRBC stroma antiserum, the IgG fraction (Cappel Laboratories, Downington, Pennsylvania), or rabbit anti-Chang cell antiserum, prepared as previously described (Hunninghake & Fauci, 1976a), were used. The dilution of antiserum in both assays was 10^{-3}, which was shown in previous studies to produce optimal cytotoxicity in these assays. For the MICC assays, PHA (Welcome Research Laboratories, Bechenham, Kent, England) was added to cultures to obtain a final concentration of 10 μg/ml, which was the previously described optimal concentration for this assay. Various effector to target cell (E : T) ratios were used. An E : T ratio of 100 : 1 resulted in a maximal release of ^{51}Cr from the target cells in each assay, in this and previous studies (Hunninghake & Fauci, 1976a). The percentage ^{51}Cr released from the target cells into the supernatant was determined as previously described (Hunninghake & Fauci, 1976b). The degree of cytotoxicity was expressed as the percentage ^{51}Cr release in the presence of PHA or anti-target cell antiserum minus the percentage ^{51}Cr release in the absence of PHA or antitarget cell antiserum. The spontaneous release of ^{51}Cr from the target cells during the 4 hr assay in the absence of effector cells was approximately 5% for the SRBC targets and 20% for the Chang cell targets.

RESULTS

Characteristics of the blood and tonsil mononuclear cell suspensions

The percentages of lymphocytes and monocytes in the blood and tonsil mononuclear cell suspensions are shown in Table 1. The blood cell suspensions contained $22 \pm 3{\cdot}9\%$ monocytes, whereas the tonsil cell suspensions contained only $2{\cdot}5 \pm 0{\cdot}3\%$ monocytes. The percentages of various mononuclear cell subpopulations in the blood and tonsil, measured by surface markers, are also shown in Table 1. The percentages of cells forming rosettes with SRBC (T lymphocytes) were significantly higher in the blood than in the tonsil ($P < 0{\cdot}001$). In contrast, the tonsil cell suspensions contained significantly greater percentages of cells forming EAC rosettes ($P < 0{\cdot}001$). The percentages of cells forming rosettes with 7S EA were not significantly different in each group ($P < 0{\cdot}5$). The percentages of cells bearing Fc receptors for 7S Ig were also determined using ox cells coated with IgG anti-ox cell antibody, as described above, and the results were the same.

TABLE 1. Percentages of various mononuclear cell subpopulations in the blood and tonsil mononuclear cell suspensions

Cells	E*	EAC†	7S EA‡	Lymphocytes	Monocytes§	Others¶
Blood						
Mean**	61·0	17	24	76	22	2
s.e.m.	1·8	1·5	2·7	3·7	3·9	0·8
Tonsil						
Mean††	34	55	22·0	92	2	6
±s.e.m.	1·9	1·6	1·6	0·6	0·3	±0·4

* Cells forming rosettes with SRBC.
† Cells forming rosettes with SRBC coated with IgM anti-SRBC antibody and complement.
‡ Cells forming rosettes with SRBC coated with IgG anti-SRBC antibody.
§ Identified by morphology and neutral esterase stains.
¶ Includes polymorphonuclear leucocytes, basophils and plasma cells.
** Mean of sixteen separate determinations.
†† Mean of thirty-eight separate determinations.

Comparison of the ADCC and MICC capacity of blood and tonsil mononuclear cells

ADCC and MICC by blood and tonsil mononuclear cells against Chang cell targets are shown in Table 2. Blood mononuclear cells were capable of functioning as effector cells in the ADCC assay; however, tonsil cells, which contained an identical proportion of 7S Fc receptor-bearing cells, were incapable of killing in this assay. In separate experiements, tonsil mononuclear cells were incubated for 2 and 18 hr at 37°C followed by multiple washings prior to their use in the cytotoxicity assays. These pre-incubated cells were also incapable of mediating ADCC against Chang liver cells. T lymphocytes have been previously reported to be the sole effectors against Chang cells in the MICC assay (Nelson *et al.*, 1976b). In the present study, both blood and tonsil cell suspensions were capable of killing Chang cells in the MICC assay and the cytotoxic capacity of each cell suspension was roughly proportional to the percentage of T lymphocytes in that cell suspension, as determined by the number of effector cells necessary to mediate 20% killing of target cells. Table 3 demonstrates the ability of blood and tonsil mononuclear cell suspensions to function as effector cells against SRBC in the ADCC and MICC assays. Monocytes, but not lymphocytes, have been previously shown to be the primary mononuclear effector

TABLE 2. Percentage ^{51}Cr release* from chang liver cell targets by blood and tonsil mononuclear cells in ADCC and PICC assays

	ADCC†; with an E : T ratio of:				MICC‡; with an E : T ratio of:			
Cells	12·5 : 1	25 : 1	50 : 1	100 : 1	12·5 : 1	25 : 1	50 : 1	100 : 1
Blood								
Mean§	3	10	24	34	8	17	30	49
s.e.m.	0·6	2·6	4·9	5·8	1·9	3·5	4·2	9·1
Tonsil								
Mean§	0	4	0	2	2	10	16	23
s.e.m.	0	1·9	0	1·2	0·6	2·1	5·7	3·2

* Assay done at 4 hr.
† Dilution of anti-Chang cells antibody was 10^{-3}.
‡ Concentration of PHA was 10 μg/ml.
§ Mean of four separate experiments, each done in triplicate.

TABLE 3. Percentage ^{51}Cr release* from sheep red blood cell targets by blood and tonsil mononuclear cells in the ADCC and PICC assays

Cells	ADCC†	PICC‡
Blood		
Mean§	45	57
s.e.m.	8·7	6·4
Tonsil		
Mean§	7	8
s.e.m.	1·6	1·8

* Assay done at 4 hr. Effector : target cell ratio was 100 : 1.

† Dilution of anti-SRBC antibody was 10^{-3}.

‡ Concentration of PHA was 10 μg/ml.

§ Mean of four separate experiments, each done in triplicate.

cell in both the ADCC and MICC assays against this target cell (Hunninghake & Fauci, 1976a). Blood mononuclear cells gave high levels of cytotoxicity, while the tonsil cell suspensions manifested low levels of killing. These results are consistent with the relative percentages of nomocytes in the respective cell suspensions (Table 1).

DISCUSSION

It has been clearly demonstrated in previous studies that ADCC against any type of target cell requires the presence on the target cell of antibody with intact Fc regions which bind to Fc receptors in the effector cell surface (Perlmann, Perlmann & Wigzell, 1972; MacLennan, 1972; Trinchieri *et al.*, 1973). In addition, Nelson *et al.* (1976b) have shown that ADCC against Chang liver cells is mediated solely by SIg-negative, E rosette-negative, Fc receptor-bearing lymphocytes. These studies, however are in conflict with those of Brier, Chess & Schlossman (1975), who found ADCC effector cells in both SIg-positive and SIg-negative lymphocyte populations obtained by Sephadex anti-Fab fractionation of human peripheral blood lymphocytes. In the present study, there was substantial killing of antibody-coated Chang liver cells by blood lymphoid cells, while tonsil lymphoid cells manifested poor killing in this assay. This striking discrepancy can not be explained by an absence of Fc receptor-positive lymphocytes in the tonsil mononuclear cell suspensions, since the relative proportions of Fc-positive cells in tonsil and blood mononuclear cell suspensions were very similar. These findings suggest that a distrinct subpopulation of the Fc-positive lymphocyte population, capable of mediating ADCC, is present in blood lymphocyte suspensions, but is markedly reduced in numbers, or absent, in the tonsil mononuclear cell suspensions. An alternative explanation for the impaired ADCC of the tonsil lymphocytes might be the presence of immune complexes bound to the lymphocyte surface, which would impair the cytotoxic effector function of these cells by blocking the Fc receptors. This is unlikely, however, because tonsil lymphoid cells incubated up to 18 hr at 37°C followed by multiple washings showed similar levels of killing of antibody-coated Chang liver cells when compared to non-incubated controls. These findings are similar to those reported by Cordier *et al.* (1976). These authors, however, attributed the reduced ADCC in tonsil mononuclear cells to a markedly reduced number of lymphoid cells bearing Fc receptors. In the present study, the percentages of tonsil and blood lymphoid cells bearing Fc receptors were found to be similar. They were determined using two different 7S EA reagents which gave identical results. The percentage of mononuclear cells in the tonsil bearing Fc receptors

could not be attributed to the presence of monocytes, since the percentage of monocytes in these suspensions, as determined by morphology and non-specific esterase staining, was 2%. In the study by Cordier *et al.* (1976), the tonsil mononuclear cells were passed over nylon wool columns prior to the 7S EA assay. In our own studies (not shown), this procedure markedly reduced the number of Fc-bearing tonsil lymphoid cells. In support of the observations in the present study is the recent study by Ryunel-Dagoo, Möller & Waterfield (1977), who found substantial numbers of Fc-positive lymphocytes in human adenoid tissue.

Nelson *et al.* (1976b) have shown that MICC against Chang liver cells is mediated by SIg negative, E rosette-positive lymphocytes. In the present study, the mitogen-induced killing of these target cells by either tonsil or blood lymphoid cells was roughly proportional to the numbers of T lymphocytes in each of these mononuclear cell suspensions. It is likely, therefore, that the difference in the mitogen-induced killing of Chang liver cells by tonsil lymphoid cells when compared to blood lymphoid cells is merely a result of a lesser proportion of T cells in the tonsil mononuclear cell suspensions. In addition, the inability of tonsil mononuclear cells to kill antibody- or mitogen-coated SRBC is probably a result of the relatively low numbers of monocytes in these cell suspensions.

The tissue distribution of lymphoid cells capable of mediating lysis of antibody-coated target cells has been examined in the rat (MacLennan & Harding, 1970), mouse (Van Boxel *et al.*, 1973) and man (O'Toole *et al.*, 1977). These studies noted a lack of reactivity in lymph node lymphocytes as compared to spleen and blood. We have previously demonstrated that effector cells capable of mediating ADCC against Chang liver cells were not present in guinea-pig bronchial-associated lymphoid tissue (BALT), whereas effector cells capable of mediating MICC against Chang cells were present in these mononuclear cell suspensions (Hunninghake & Fauci, 1976a). Nelson *et al.* (1976a) have noted similar findings in lymphocytes from guinea-pig gut-associated lymphoid tissue (GALT). These studies suggest that an absence of effector cells capable of mediating ADCC may be a common feature of lymphoid cell suspensions isolated from various endothelial cell surfaces. In each of these studies, lymphoid cell suspensions from BALT, GALT and tonsil have demonstrated intact MICC. In addition, these studies lend further support to previous findings (Nelson *et al.*, 1976b) that ADCC and MICC against Chang liver cells were mediated by different effector cells.

Thus the present study demonstrates that in certain types of cytotoxicity assays, the cytotoxic capabilities of mononuclear cells from different organs are a direct reflection of the relative proportions of effector cells mediating the cytotoxicity against a given target. However, in the case of cytotoxicity mediated by an Fc receptor-bearing, non-phagocytic, non-adherent lymphoid cell, there is a clear discrepancy between the relative proportions of Fc receptor-bearing lymphoid cells in different organs and the relative levels of cytotoxicity. This can be explained either by alterations in the absolute numbers of a distinct subset of Fc receptor-bearing cells in different lymphoid organs, or by the presence of this population of Fc receptor-bearing lymphoid cells in different stages of maturity in various organs and reflected by different cytotoxic capabilities.

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