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The antibody-independent cytotoxic activity of normal circulating human leucocytes

II. FAILURE TO DEMONSTRATE EFFECTOR CELL–TARGET CELL INTERACTION AND TARGET CELL SPECIFICITY OF THE CIRCULATING CYTOTOXIC-ENHANCING FACTOR

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Summary. The naturally-occurring antibody-independent cellular cytotoxic activity (NOCC) of normal circulating human monocytes and neutrophils was investigated employing a number of erythrocytes and the K-562 cell line as target cells simultaneously. The identity of the effector cell(s) was shown to be dependent upon or be a function of the type of target cell selected for the assay system.

Abbreviations: M199, medium 199 (Microbiological Associates, Bethesda, Md.); NHS, normal human serum; FCS,: foetal calf serum (Microbiological Associates); culture medium, M199 fortified with penicillin (100 units per ml), streptomycin (100 μg per ml) and NHS (to a final concentration of 1%); HBSS, Hanks's balanced salt solution (Microbiological Associates); HAGG, human aggregated gammaglobulin; RFC, rosette-forming cell; E-RFC, rosette formed with sheep erythrocytes (E); EA-RFC, rosette formed with antibody (A)-sensitized ox erythrocytes (E) (EA); EAC-RFC, rosette formed with C'3 (C) fixed onto EA (EAC); ADCC, antibody-dependent cellular cytotoxicity; NOCC, naturally-occurring cellular cytotoxicity; SLMC, spontaneous lymphocyte-mediated cytotoxicity; SRBC, sheep erythrocytes; ORBC, ox erythrocytes; CRBC, chicken erythrocytes; HuRBC, human erythrocytes; RRBC, rabbit erythrocytes; CI, cytotoxicity index; SR, spontaneous release; HRBC, horse erythrocytes; HSA, human serum albumin.

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A number of erythrocyte targets (rabbit, horse, sheep and ox erythrocytes) were lysed to varying degrees by neutrophils and monocytes and not by lymphocytes. Irrespective of the red blood cell (RBC) target, the effector monocyte invariably possessed receptors for both C'3 and the Fc of IgG. In contrast, the cytotoxic cells using the K-562 target cell were lymphocytes. Monocytes and neutrophils were inactive.

The cytotoxic-enhancing activity in normal human serum exhibits specific and non-specific properties which suggests that more than one factor is involved. With respect to the monocyte cytotoxic cells, only the rabbit erythrocytes could totally absorb the serum factor in a specific fashion. Absorption of the serum with horse, sheep or ox erythrocytes resulted in a significant loss of potentiating activity with respect to all of the erythrocyte targets but a more marked loss of activity using the absorbing erythrocytes as targets. With respect to the polymorphonuclear leucocyte effector cells, only the rabbit RBC were capable of specifically absorbing out the cytotoxic-enhancing factor present in the normal human serum. Absorption of the serum with sheep, horse or ox RBC resulted in total cross-absorption of the enhancing factor. Chicken and human RBC, which do not serve as targets for the NOCC assay, could not absorb out the cytotoxic-enhancing factor with respect to any of the target erythrocytes. The composition of the soluble serum factor(s) is under current investigation but it is

not an immunoglobulin since pure serum albumin can substitute for normal serum in the NOCC assay.

The mechanism of erythrocyte lysis by the cytotoxic monocyte was investigated. Mononuclear cells were incubated with target cell monolayers and with target cells under optimal rosetting conditions. No interaction between the effector and target cells could be detected. The monocytes did not adhere to the target cell monolayer nor did they form rosettes with the target cells. Thus, the results fail to corroborate or support the assumption that the cytotoxic activity of the monocyte is dependent upon conventionally-detectable receptors. Erythrophagocytosis was not observed to any significant degree under the assay conditions used. Therefore, the nature of the interaction between the cytotoxic monocyte and the erythroid target cell which results in lysis of the target cell remains to be elucidated.

INTRODUCTION

In the accompanying communication, it was demonstrated that the rabbit erythrocytes (RRBC) are lysed in an antibody-independent non-phagocytic pathway (NOCC) by normally-circulating human monocytes and neutrophils (Banerjee, Fernando, Sklar & Richter, 1981). Lymphocytes were inactive in this assay. The cytotoxic monocytes possess receptors for both C'3 and the Fc of the IgG molecule. The NOCC cytotoxic reaction was shown to be distinct from the antibody-dependent cell-mediated cytotoxic (ADCC) reaction. The lysis of the RRBC target cell could not be attributed to secreted soluble factors nor erythrophagocytosis.

The questions which were addressed in this investigation are: (i) can erythrocytes other than RRBC function as target cells in the NOCC cytotoxic reaction; (ii) is the identity of the effector cell a function of the target cell? In other words, are the effector cells different when related and unrelated target cells are utilized simultaneously with the same cytotoxic cells; and (iii) do the NOCC cytotoxic cells possess receptors directed toward the target cells which can be detected by conventional techniques?

METHODS AND MATERIALS

The techniques utilized for the isolation of human circulating mononuclear cells, polymorphonuclear leucocytes, monocyte-depleted mononuclear cells, E, EA, and EAC rosetted cells and receptor (E, Fc and C'3) cell-depleted mononuclear cells have been described in the accompanying paper (Banerjee *et al.*, 1981).

Heparinized rabbit, horse, chicken, ox and sheep blood were obtained fresh weekly from Qualicum Laboratories, Ottawa. Human erythrocytes (HuRBC) were isolated from the blood of the individuals who served as the donors of the cytotoxic cells.

The K-562 target cells were kindly provided by Dr Hugh Pross, Ontario Cancer Foundation, Kingston General Hospital, Kingston, Ontario. They were maintained in suspension culture at 37° in 5% CO_2 in air until used.

The culture medium used in the assay utilizing the RBC target cells is Medium 199 (Microbiological Associates, Bethesda, Md.) fortified with heated (56°, 60 min) normal human serum (NHS, final concentration 1%), penicillin (100 units per ml) and streptomycin (100 μg per ml). The culture medium used in the assay utilizing the K-562 target cells is Medium RPMI 1640 (Microbiological Associates) fortified with heated (56°, 60 min) foetal bovine serum (final concentration 10%), penicillin (100 units per ml) and streptomycin (100 μg per ml).

Preparation of ^{51}Cr-labelled target cells and cytotoxicity assay

The preparation of ^{51}Cr-labelled target cells, the assay used and the calculation of the cytotoxic activity are described in the accompanying paper (Banerjee *et al.*, 1981).

Elimination of cells with affinity for the RRBC target cells

Adherence of cells to an RRBC monolayer. Poly-L-lysine (50 μg/ml) was added to a plastic Petri dish in a volume sufficient to fully cover the bottom surface. The Petri dish was then incubated for a minimum of 30 min at 37° in 5% CO_2 in air and washed three times in saline. A 5% suspension of RRBC made up in M199 was added in sufficient volume to just cover the bottom surface. The plate was incubated for 30 min and then washed in PBS until only a monolayer of erythrocytes could be seen under the inverted microscope. The mononuclear effector cells (2×10^6 cells per ml) were added in a volume sufficient to cover the bottom of the plate. The dish was then incubated at 37° for 120 min and the non-adherent cells were gently pipetted off. The adherent cells were dislodged using more vigorous

Table 1. The NOCC cytotoxic activity of different populations of human circulating cells assayed against different ^{51}Cr-labelled erythrocytes as targets

	The NOCC cytotoxic index (CI) of circulating cells with respect to the following ^{51}Cr-labelled erythrocyte targets (effector to target cell ratio is 5:1)																	
	Rabbit RBC			Sheep RBC			Ox RBC			Horse RBC			Chicken RBC			Human RBC		
Exp. no.	MC*	MDMC†	P‡	MC	MDMC	P	MC	MDMC	P	MC	MDMC	P	MC	MDMC	P	MC	MDMC	P
I	61	6	103	28	6	61	24	0	17	49	12	92	5	2	9	4	0	0
II	77	6	100	22	5	70	16	0	34	60	2	91	1	0	28	0	0	2
III	62	2	98	32	2	11	11	1	21	61	3	93	2	0	3	0	0	1
IV	64	4	91	26	ND§	61	17	ND	62	53	ND	83	3	ND	34			
V	51	3	93	53	ND	30	21	ND	12	100	ND	98	5	ND	8			

* MC, mononuclear cells at interface following Ficoll-Hypaque centrifugation of whole blood.
† MDMC, monocyte-depleted mononuclear cells.
† P, polymorphonuclear cells in the pellet following Ficoll-Hypaque centrifugation of buffy coat cells.
§ ND, not done.

agitation. Both the non-adherent and adherent cells were washed twice in saline, resuspended in medium and counted.

Elimination of mononuclear cells which form rosettes with RRBC. The mononuclear cells were incubated with RRBC in the same proportions and under the same conditions as they are incubated with E, EA and EAC to form optimal numbers of E, EA and EAC rosettes. The procedure is identical to that described in a previous paper (Banerjee *et al.*, 1981).

Each protocol was repeated a minimum of five times with the majority of experiments repeated ten to fifteen times.

RESULTS

As can be seen in Table 1, in which are presented the results of five of fifteen experiments carried out, rabbit (RRBC) and horse (HRBC) erythrocytes were consistently lysed almost equally well by the normally-circulating mononuclear cells and polymorphs. Sheep (SRBC) and ox (ORBC) erythrocytes were lysed to a much lesser degree. Chicken (CRBC) erythrocytes were lysed only by the polymorphonuclear cells of about 25%–30% of the individuals tested while neither autologous nor allogeneic human erythrocytes were lysed. In the cases of the RRBC, HRBC, SRBC and ORBC, the mononuclear cytotoxic cell was identified as the monocyte since removal of carbonyl iron ingesting cells from the mononuclear cell suspension consistently eliminated both the monocyte and the cytotoxic activity of the remaining cells (Table 1). As was shown previously with the RRBC target cells, the T cells exhibit very little or no cytotoxic activity with respect to any of the erythrocyte target cells (results not presented in Table).

With respect to the SRBC, ORBC, RRBC and HRBC target cells, the cytotoxic activity displayed by the mononuclear cells is a property of the C'3 and Fc (IgG) receptor-bearing monocytes since mononuclear cells depleted of either C'3 or Fc (IgG) receptor-bearing cells possessed no cytotoxic activity (results not presented in the Tables).

Circulating mononuclear cells were very effective in optimally lysing the K-562 target cells in the total absence of serum; however, spontaneous release of ^{51}Cr was considerable in serum-free RPMI 1640 (20%–35%) as compared with that in RPMI 1640 fortified with foetal bovine serum in a final concentration of 10% (spontaneous release 5%–10%). In experiments using the serum-fortified medium RPMI 1640, it was demonstrated that polymorphonuclear leucocytes possess no cytotoxic activity for the K-562 target cell and that the lymphocyte and not the monocyte is the cytotoxic monunuclear cell (Table 2).

The human serum used to supplement the culture medium in the cytolytic assay was absorbed with the different target cells before its addition to the culture medium in order to establish whether the serum factors which potentiate, enhance or promote the cytolysis of the target cells by the circulating effector mononuclear cells are target cell specific (antibodies?) or non-specific. Only the RRBC could specifically absorb the cytotoxic potentiating factor (s) from the serum with respect to the RRBC target (Table 3). The HRBC, SRBC and ORBC were capable of removing the enhancing factor in a mixed manner, that is a specific and non-specific manner since the cytotoxic-enhancing activity of the serum absorbed with any one of these erythrocytes was almost totally removed with respect to the absorbing erythrocyte and it was markedly reduced with respect to the other RBC targets insofar as the mononuclear effector cells are concerned (Table 3).

With respect to the polymorphonuclear effector cells, only the rabbit erythrocytes could totally absorb the cytotoxic-enhancing factor. The sheep, ox and horse erythrocytes absorbed the factor in an appar-

Table 2. The NOCC cytotoxic activity of normal human circulating lymphocytes, monocytes and polymorphonuclear leucocytes with respect to K-562 and RRBC target cells

	The NOCC cytotoxic activity of the following human circulating cells with respect to ^{51}Cr-labelled RRBC and K-562 target cells (effector to target cell ratio is 5:1)					
	RRBC target cells			K-562 target cells		
Exp. no.	MC*	MDMC†	P‡	MC	MDMC	P
I	54	2	91	14	14	5
II	52	8	84	25	30	4
III	57	1	83	29	27	0

* MC, unfractionated mononuclear cells.

† MDMC, macrophage-depleted mononuclear cells.

§ P, polymorphonuclear leucocytes.

Table 3. The capacity of human serum to support the NOCC cytotoxic activity of circulating human cells following absorption of the serum with the different erythrocytes

Serum absorbed with following erythrocytes	The NOCC cytotoxic index of mononuclear and polymorphonuclear effector cells with respect to the following ^{51}Cr-labelled erythrocytes, in the presence of erythrocyte-absorbed serum*							
	Mononuclear effector cells†				Polymorphonuclear effector cells†			
	Rabbit	Sheep	Ox	Horse	Rabbit	Sheep	Ox	Horse
Nil	87	53	35	100	93	68	63	100
Rabbit	5	25	11	10	9	2	5	0
Sheep	74	0	18	30	100	0	4	2
Ox	69	14	4	33	80	3	2	2
Horse	100	20	14	2	96	0	2	0
Chicken	100	45	37	95	100	50	58	100
Human	95	46	33	95	99	48	62	100

* Effector to target cell ratio is 5:1.

† Cells at interface following centrifugation of whole blood in a discontinuous Ficoll-Hypaque density gradient.

‡ Pelleted cells following centrifugation of buffy coat cells in a discontinuous Ficoll-Hypaque density gradient.

ently non-specific manner since activity was totally absorbed with respect to the SRBC, ORBC and HRBC targets with each of these erythrocytes, but not with respect to the RRBC, irrespective of the target erythrocyte used (Table 3). The CRBC and HuRBC which cannot serve as targets in the NOCC assay could not absorb the potentiating factors with respect to any of the RBC target cells (Table 3).

Attempts were made to demonstrate receptors on the cytotoxic monocytes for the RRBC target cells on the basis of the ability of the cytotoxic cells to (i) form rosettes with the target cells and (ii) adhere to target cell monolayers. The cells 'depleted' of rosetting cells (the cells at the interface following centrifugation in Ficoll-Hypaque after incubation of the cells with RRBC under optimal rosetting conditions) were as cytotoxic as the untreated cells (Table 4). The cells which did not adhere to the RRBC monolayer were as

Table 4. The failure to detect rosette formation between the human circulating NOCC mononuclear effector cells and the RRBC target cells

Exp. no.	The NOCC cytotoxic activity (CI) of untreated and RRBC rosetting cell-depleted mononuclear cells with respect to ^{51}Cr-labelled RRBC and HRBC target cells (effector to target cell ratio is 10:1)			
	Untreated mononuclear cells		'Rosetting cell'-depleted mononuclear cells	
	^{51}Cr-RRBC	^{51}Cr-HRBC	^{51}Cr-RRBC	^{51}Cr-HRBC
I	64	26	70	19
II	67	28	69	37
III	57	24	61	23

Table 5. The failure to detect adherence of the human circulating NOCC mononuclear effector cells to target RRBC monolayers

	The NOCC cytotoxic activity (CI) of untreated and RRBC-adherent cell-depleted circulating mononuclear cells with respect to ^{51}Cr-labelled RRBC and HRBC target cells (effector to target cell ratio is 10:1)			
	Untreated mononuclear cells		Mononuclear cells depleted of adherent cells	
Exp. no.	^{51}Cr-RRBC	^{51}Cr-HRBC	^{51}Cr-RRBC	^{51}Cr-HRBC
I	64	26	49	19
II	67	28	63	25
III	57	24	53	27

cytotoxic with respect to the RRBC target cells as were the untreated cells (Table 5).

DISCUSSION

In a previous investigation (Banerjee *et al.*, 1981), it was demonstrated that subclasses of normally-circulating monocytes and neutrophils are capable of lysing rabbit erythrocytes in culture. This cytolytic reaction was independent of erythrophagocytosis, antibodies and soluble mediators. The cytotoxic monocyte was found to possess receptors for both Fc (IgG) and C'3. Lymphocytes were totally devoid of cytotoxic activity. However, the significance of the identification of the cytotoxic cell may be of dubious value in view of the conflicting results obtained by numerous investigators engaged in defining the identity of the naturally-occurring antibody-independent cytotoxic cell(s). Thus, the circulating human cytotoxic cell has variously been identified as a T cell with or without receptors for Fc (IgG) (West, Cannon, Kay, Bonnard & Herberman, 1977; Coates & Crawford, 1977; Kall & Koren, 1978; Pape, Moretta, Troye & Perlmann, 1979; Potter & Moore, 1979; Ortaldo, Bonnard, Kind & Herberman, 1979; Herberman, Nunn, Holden, Staal & Djeu, 1977; Herberman, Nunn & Holden, 1978; Bolhuis, Schuit, Nooyen & Ronteltap, 1978), a non-T cell (Cooper, Hirsen & Friou, 1977; Shellam, 1977; Pross, Gupta, Good & Barnes, 1979; Kiessling, Petranyi, Karre, Jondal, Tracey & Wigzell, 1976), a monocyte or phagocyte cell (Banerjee *et al.*, 1981; Muchmore, Decker & Blaese, 1977, 1979a, 1979b; Stott, Probert & Thomas, 1975; Horwitz, Kight, Temple & Allison, 1979; Melson & Seljelid, 1973), a non-B cell (Cooper *et al.*, 1977; Kiessling, Klein, Pross & Wigzell, 1975; Kiessling *et al.*, 1976; Nelson, Bundy & Strober, 1977; Gidlund, Ojo, Orn, Wigzell & Murgita, 1979) with receptors for C'3 (de Vries, Cornain & Rumke, 1974) or C'3 and Fc (IgG) (Jondal & Pross, 1975; Pross, Baines & Jondal, 1977), or a non-B non-T 'Null' cell with receptors for Fc (IgG) but not for C'3 (Pross *et al.*, 1979; Kiessling *et al.*, 1976; Kiuchi & Takasugi, 1976; Galili & Schlesinger, 1978; Herberman, Nunn, Holden & Lavrin, 1975; Hersey, Edwards, Edwards, Adams, Milton & Nelson, 1975; Parrillo & Fauci, 1978). Although the majority of investigations have implicated a cell with receptors for Fc (IgG) (Pross *et al.*, 1979; Kiessling *et al.*, 1976; Kiuchi & Takasugi, 1976; Galili & Schlesinger, 1978; Herberman *et al.*, 1975; Hersey *et al.*, 1975; Parrillo & Fauci, 1978; and Möller, 1979), reference has been made to naturally-occurring cytotoxic cells devoid of surface receptors for Fc (IgG) (Bakacs, Gergely & Klein, 1977; Bolhuis *et al.*, 1978). One interpretation for these conflicting and apparently irreconcilable findings is that the host is genetically programmed to synthesize numerous types of cytotoxic cells, each endowed with a distinct specificity with regard to a particular target cell. Therefore, cytotoxic cells may be considered to be unipotent, capable of lysing only a specific target cell. Thus the identification of a distinct cytotoxic cell is dependent upon the target cell used in the assay. In this regard, it is instructive to note that investigators utilize many different target cells in the NOCC or natural killer (NK) assays. In point of fact, in excess of thirty

Table 6. Target cells used in the NOCC assay

Proper name	Origin
Hela cell	Human cervical carcinoma
Chang cell	Human liver
P-815	Methylcholanthrene-induced mastocytoma, maintained in ascites form
K-562	Human chronic myelogenous leukaemic cell line
EL-4	Methylcholanthrene-induced thymoma of mouse origin, maintained in ascites form
YAC-1	Maloney leukaemia virus-transformed mouse T-lymphoma cell
FBL-3	Lymphoma cell line
Human foetal fibroblasts	
Erythrocytes	Sheep, chicken, horse, rabbit
Molt-4	Human acute lymphocytic leukaemia cell
RAJI	Burkitt lymphoma cell line
RPMI 6410	Human myelogenous leukaemia cell line
CLA-4	Human B-cell line
T-24	Human bladder carcinoma
MDA-MB231	Human mammary carcinoma
IGR-3	Human malignant melanoma

different target cells—malignant, foetal and normal cell lines, erythrocytes and normal lymphoid parenchymal cells, are currently being utilized by different investigators. The most frequently used target cells and their derivation are presented in Table 6. It may therefore be that the confusion surrounding the identity of the effector cells stems from the anticipation, based more on hope than on scientifically validated evidence, that the effector cells for all of the different target cells have similar, if not identical, characteristics. The reality, as stated above, may be that different effector cells are generated with respect to different target cells. To verify the heterogenous nature of the normally-circulating cytotoxic cells, it must be shown that these cells, obtained from a single individual, are indeed different when confronted simultaneously under identical culture conditions with a number of different target cells. In other words, it is necessary to demonstrate that factors other than the nature of the target cell (i.e. different cell isolation and culture conditions in different laboratories) are not instrumental in the identification of the cytotoxic cell(s).

This objective was realized to a considerable extent in this investigation. Four erythrocyte targets, RRBC, HRBC, ORBC and SRBC, were consistently lysed to different degrees by the human cytotoxic mononuclear and polymorphonuclear cells. The lymphocytes were inactive. The cytotoxic monocytes were invariably shown to bear receptors for C'3 and Fc irrespective of the RBC target used. On the other hand, the cytotoxic cells directed toward the K-562 cells were shown to be lymphocytes; monocytes and polymorphs were consistently inactive. Thus, the effector cells of a single individual do indeed vary with the target cells.

The second objective was to determine whether the cytotoxic cells can be shown to possess detectable surface receptors for determinants on the target cells, much like the 'K' cells which mediate the ADCC reaction possess receptors for the Fc of IgG antibodies bound to the target cells. This objective proved to be more elusive and not attainable with the techniques employed. The basic assumption was that the lysis of the RRBC target cell by the monocyte cytotoxic cell is preceded by a non-random interaction between these two cells for a finite period of time, involving receptors on the cytotoxic cell and specific determinants on the target cell. It was therefore anticipated that the cytotoxic cells would adhere to a target cell monolayer or that they would form rosettes with the target RRBC, much like Fc receptor-bearing ADCC cytotoxic cells, T cells and C'3 receptor-bearing cells form rosettes with EA, E and EAC, respectively. However, using the identical conditions which facilitate the formation of the maximum numbers of E, EA and EAC rosettes, the NOCC cytotoxic cells did not form any detectable rosettes with the RRBC. Longer incubation of the cytotoxic monocytes with the target cells failed to encourage an interaction of sufficient magnitude to be detected by rosetting procedures.

The cytotoxic monocytes failed to adhere to the RRBC target cell monolayer, even when only threshold numbers of these cells were co-incubated with the target cell monolayer for 1–2 hr. The cytotoxic activity was displayed by the non-adherent cells. Therefore, the mechanism of interaction between the cytotoxic monocytes and the target erythrocytes would not appear to be dependent upon receptors on the monocyte (directed toward determinants on the target cell) detectable by conventional assays. Previous investigation (Banerjee *et al.*, 1981) could not implicate a soluble mediator(s) released from the effector cells, nor was erythrophagocytosis observed to any significant degree (Banerjee *et al.*, 1981). The

cellular interactions and mechanism of target cell lysis by the NOCC pathway cannot therefore be elucidated on the basis of current findings.

An interesting finding was that only the erythrocytes which functioned as targets in the NOCC assay, rabbit, horse, sheep and ox, could absorb the cytotoxic enhancing factor(s) in the human serum used to supplement the culture medium. Chicken and human erythrocytes, which are not lysed in the NOCC assay, are incapable of absorbing the serum factor (Table 3). Furthermore, the absorbed sera did not facilitate or augment the NOCC cytotoxic activity of the monocytes and polymorphonuclear cells to the same degree. With respect to the rabbit RBC targets and the monocyte effector cells, only the rabbit RBC could absorb out the cytotoxic enhancing factor; absorption of the serum with any of the other RBC did not adversely affect the capacity of the serum to sustain optimal monocyte-mediated NOCC lysis of the rabbit RBC. With respect to the horse, sheep and ox RBC targets and monocyte effector cells, such specificity associated with absorption with the RRBC was lacking. Serum absorbed with any of these three RBC displayed a markedly diminished capacity to enhance the monocyte-mediated NOCC lysis with respect to any of these three RBC targets, but more striking was the total loss of the capacity to enhance the NOCC lysis with respect to the absorbing erythrocyte (Table 3). Furthermore, even serum absorbed with rabbit RBC displayed minimal capacity to sustain NOCC lysis of the horse, sheep or ox RBC target cells. The results with the polymorphonuclear cytotoxic cells were in some respects similar but in other respects more extreme than those obtained with the monocytes. With respect to the rabbit RBC targets, only the serum absorbed with rabbit RBC lost its ability to sustain the NOCC cytotoxic activity. However, with respect to the horse, sheep and ox RBC targets, absorption of the serum with any of the four RBC (rabbit, horse, sheep or ox) resulted in total absorption of the cytotoxic enhancing factor (Table 3). The RBC-absorbed sera could not sustain the NOCC lysis of any of the RBC targets by the polymorphonuclear effector cells. Therefore, as with the monocyte effector cells, the absorption of the serum with the rabbit RBC imparts a degree of specificity to the assay system since only the rabbit RBC could absorb out the cytotoxic enhancing factor with respect to rabbit RBC targets. The non-specific absorptive capacities of the horse, sheep and ox RBC were most pronounced when the polymorphonuclear leucocytes were assessed for cytotoxic activity with respect to the horse, sheep or ox RBC targets.

The results with the monocyte effector cells suggest that the NOCC cytotoxic-enhancing factors in normal human serum are heterogeneous, that there are at least two in number, one specific for the target cell and one present on all erythrocyte target cells with the possible exception of the rabbit RBC. It may be necessary to postulate a third, non-specific, cytotoxic-enhancing factor to account for the loss of neutrophil-mediated NOCC activity in the presence of absorbed serum under conditions where the effector monocytes exhibit significant cytotoxic activity. Current investigations are aimed at resolving this question.

There is therefore a general but not an absolute relationship between an erthrocyte which can function as a target cell and its ability to absorb the cytotoxic enhancing factors in normal serum which are required for the expression of maximum NOCC cytotoxic activity by the effector monocytes and neutrophils. These factors cannot be considered to be antibodies in view of the fact that serum albumin and agamma serum can substitute for serum in the NOCC assay (Banerjee *et al.*, 1981) and the non-specific manner whereby they are absorbed. Recently, Muchmore *et al.* (1979a, b) described a heat-labile non-antibody factor in human plasma which 'armed' T cells and monocytes to enable them to express cytotoxic activity with respect to chicken erythrocyte, but not allogeneic erythrocyte, targets. Whether the cytotoxic enhancing factors described here are similar or identical to that described by Muchmore *et al.* (1979a, b) remains to be elucidated.

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