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Synergistic effect of blending IgG1 and IgG3 monoclonal anti-D in promoting the metabolic response of monocytes to sensitized red cells

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SUMMARY

Monoclonal antibodies specific for the Rh antigen D were used to sensitize red cells for use in a series of cellular assays. IgG3 anti-D was more efficient than IgG1 anti-D in promoting the attachment and lysis of red cells by human monocytes. In contrast, IgG1 anti-D was more efficient at mediating phagocytosis. The metabolic response of monocytes, measured by chemiluminescence (CL), was greater towards IgG3-sensitized red cells than IgG1-sensitized cells; however, the CL response was further increased when red cells were sensitized in antibody mixtures comprising both subclasses. Using monoclonal antibodies from five IgG1-secreting cell lines and from three IgG3-secreting cell lines, this synergistic increase was seen with 0/4 IgG1/IgG1 combinations, 0/2 IgG3/IgG3 combinations and 8/8 IgG1/IgG3 combinations. Synergism was observed only when both subclasses were present on the same red cells; mixing of IgG1-sensitized red cells with IgG3-sensitized red cells before addition to monocytes did not increase CL generation. The binding and phagocytosis of red cells by monocytes and the lysis of red cells by monocytes or lymphocytes were not greater when red cells were sensitized with IgG1 and IgG3 antibodies together compared to red cells coated with single subclasses.

Monoclonal human anti-D has been produced by culture of Epstein–Barr virus (EBV)-transformed B-lymphoblastoid cell lines derived from immunized donors (Kumpel, Poole & Bradley, 1989). There is currently much interest in the possibility of using monoclonal anti-D as a replacement for the plasma-derived anti-D immunoglobulin currently in use for prophylaxis against haemolytic disease of the newborn (Hughes-Jones, 1988). The prophylactic effect may depend, at least in part, on the ability of administered anti-D to cause the sequestration and destruction of fetal red cells by maternal macrophages (reviewed by Bowman, 1988). Since the polyclonal preparation contains both IgG1 and IgG3 anti-D, the current *in vitro* study was undertaken to determine the role of these subclasses, both individually and in combination, in monocyte-mediated red cell destruction.

Five culture supernatants containing monoclonal IgG1 anti-D (FC3, 2B6, R1D7, AB5, 1A3-1) and three containing IgG3 anti-D (CB6, 6D10, 1A3-3) (Kumpel *et al.*, 1989) were used to sensitize D-positive (OR_1R_2) red cells. The IgG1 antibodies were derived from three donors and the IgG3 antibodies from one of these donors. Sensitization levels were measured radiometrically using ^{125}I-labelled sheep anti-human IgG (Merry *et al.*, 1982). Preliminary experiments were conducted to establish the supernatant dilutions necessary to achieve different levels of cell-bound anti-D. Red cells were then sensitized in blends of the diluted supernatants such that the bound anti-D ranged from 100% IgG1 to 100% IgG3.

Binding of sensitized red cells to adherent human monocytes was determined as described elsewhere (Hadley *et al.*, 1989). Results are reported as the percentage of monocytes with one or more peripherally bound red cell. Phagocytosis of red cells was determined by incubating adherent monocytes with sensitized red cells (ratio 30 red cells to one monocyte) for 4 h at 37° in 24-well tissue culture plates (Costar, Cambridge, MA). Unbound red cells were removed by rinsing the wells with phosphate-buffered saline (PBS, pH 7·2). Peripherally bound (but not internalized) red cells were then lysed by addition of 1·0% w/v ammonium oxalate containing 2 mM Na_2 EDTA for 2 min. Thereafter cells were restored to isotonicity with PBS for 5 min, dried, fixed, stained and assessed microscopically. Results are reported as the percentage of monocytes with one or more internalized red cell. Generation of luminol-enhanced CL by monocytes during erythrophagocytosis was measured as described previously (Hadley, Kumpel & Merry, 1988). The CL response of monocytes incubated with sensitized cells was recorded for 1 hr, then compared with the CL response to unsensitized cells, and expressed as the ratio or opsonic index. Monocyte-mediated lysis (antibody-dependent cell-mediated cytotoxicity, ADCC) of ^{51}Cr-labelled red cells (Hadley *et al.*, 1989) is expressed as a percentage of the ^{51}Cr released from red cells by 1% v/v Triton X-100. Lymphocyte-mediated specific

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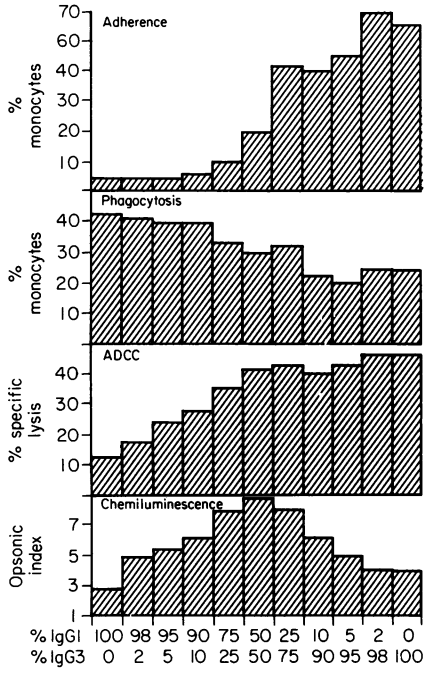

Figure 1. The interactions between human monocytes and red cells sensitized with IgG1 and IgG3 anti-D antibodies in various proportions. Results are from single representative experiments. The red cells were sensitized with 9100 IgG1 molecules per cell or 8900 IgG3 molecules per cell when sensitized in individual supernatants, and with 8660 IgG molecules per cell when sensitized with a 50/50 combination (means of triplicate determinations). The graphs show the percentage of monocytes with adherent or phagocytosed red cells, the extent of red cell lysis and the CL response in terms of opsonic index. The opsonic index for unsensitized cells was $1{\cdot}0 \pm 0{\cdot}1$ (mean $\pm$ 2 SD). The red cell:monocyte ratios used were approximately 30:1 in adherence and phagocytosis assays, 1:2 in the ADCC assay, and 10:1 in the CL assay.

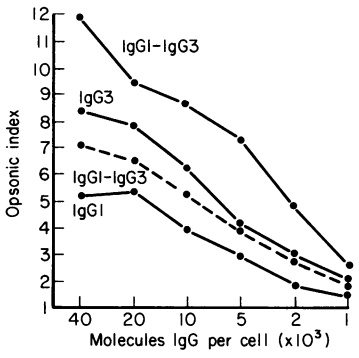

Figure 2. The increase in CL response of monocytes incubated with red cells sensitized with both IgG1 and IgG3 anti-D. Results are from a single representative experiment. The solid lines show monocyte responses to red cells sensitized with IgG1 anti-D, IgG3 anti-D, or a 50/50 blend of IgG1 and IgG3 anti-D. For comparison, the broken line shows monocyte response to a mixed population of IgG1-sensitized and IgG3-sensitized red cells.

Table 1. Lysis of red cells by lymphocytes in an ADCC assay

Ratio of sensitizing antibody	IgG1:IgG3	IgG1:Diluent
	% specific lysis	
4:0	33	33
3:1	19	25
2:2	7	8
1:3	1	2
0:4	1	−2

Antibodies used were 1A3-1 and 1A3-3. The diluent was complete culture medium.

lysis of red cells in an ADCC assay (Urbaniak, 1976) was measured using red cells presensitized at approximately 20,000 IgG molecules per red cell, and at a lymphocyte to target cell ratio of 15:1.

Figure 1 shows that the adherence and lysis of sensitized red cells by human monocytes increased with increasing proportions of IgG3 in the sensitizing mixture. Conversely, phagocytosis of red cells was greater with increasing proportions of IgG1. However, the metabolic response of monocytes (as measured by CL) was greatest when both subclasses were present in the opsonizing mixture in approximately equal proportions (Fig. 1). Quantification of cell-bound IgG showed that this increase was not due to raised levels of bound anti-D when red cells were incubated in mixtures of antibodies. This synergistic increase in the ability of red cell-bound IgG to elicit a CL response from monocytes was observed only when IgG1-containing supernatants were blended with IgG3-containing supernatants. Increased CL responses were observed in 0/4 IgG1/IgG1 mixtures, 0/2 IgG3/IgG3 mixtures and 8/8 IgG1/IgG3 mixtures. The synergistic increase in CL response was detected at levels of sensitization from approximately 30,000 IgG molecules per cell (corresponding to the approximate number of D antigen sites) to approximately 2000 IgG molecules per cell (limit of assay sensitivity). Further, increased CL responses were seen only when both subclasses were present on the same red cells (Fig. 2). Thus, mixing of red cells sensitized with only IgG1 anti-D with red cells sensitized with only IgG3 anti-D prior to their addition to monocytes did not result in levels of CL generation above the expected mean.

Lymphocyte-mediated red cell lysis was only obtained with IgG1 anti-D, and was roughly proportional to the amount of IgG1 on the red cells (Table 1).

The increase in monocyte CL response to red cells sensitized with both IgG1 and IgG3 antibodies may result from differences in the functional characteristics of these subclasses. IgG3 anti-D is more efficient at mediating the binding of red cells to monocytes and the U937 cell line via Fc receptors (Walker *et al.*, 1988; Hadley *et al.*, 1989). This is probably due to the long hinge region of IgG3 which confers upon the molecule the ability to bridge the gap between two negatively charged cells more effectively than IgG1 (Kumpel, Leader & Bradley,1988). Cell-bound IgG1, once engaged by Fc receptors, may be more efficient at promoting some of the monocyte responses which follow receptor cross-linking or immobilization. Consistent with the results of Wiener *et al.* (1988), at a red cell to monocyte ratio of 30:1, IgG1 anti-D was more efficient at promoting

phagocytosis than IgG3 anti-D. Further, the CL response of monocytes pretreated with neuraminidase to reduce their negative charge and so circumvent the steric advantage of IgG3, is greater to red cells sensitized by IgG1 than IgG3 anti-D (B. M. Kumpel and A. G. Hadley, manuscript in preparation). The ability of cell-bound IgG1 to efficiently activate monocytes may be due to its short hinge region limiting lateral mobility of membrane receptors and resulting in more effective immobilization. Thus the presence of both IgG1 and IgG3 anti-D on the same cell may result both in efficient red cell adherence and Fc receptor immobilization with increased CL generation as a consequence.

In the current study, monocyte response to sensitized red cells was measured in terms of adherence, phagocytosis, metabolic activity and red cells lysis. The synergistic effect was only observed with the metabolic response and was not detected in the rosette, phagocytosis or ADCC assays. Red cell lysis by monocytes is primarily extracellular in nature (Fleer *et al.*, 1978) and the non-oxidative mechanisms involved (Fleer *et al.*, 1979) may be triggered differently to the oxidative activity detected by CL. The absence of synergy in the phagocytosis assay was unexpected since we have previously shown the CL response is associated with, or secondary to erythrophagocytosis (Hadley *et al.*, 1988). In the lymphocyte-mediated ADCC assay, synergy with blends of IgG1 and IgG3 antibodies was not observed.

These *in vitro* findings may have significance for the formulation of a monoclonal antibody-derived preparation for Rh prophylaxis. If the functional differences between the IgG subclasses described above operate *in vivo*, then a blend of monoclonal antibodies may be necessary for maximal efficacy. IgG3 anti-D may be necessary to promote rapid clearance of fetal cells to the maternal spleen, and IgG1 may be necessary for efficient phagocytosis.

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