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Cellular origins of human polymeric and monomeric IgA: enumeration of single cells secreting polymeric IgA1 and IgA2 in peripheral blood, bone marrow, spleen, gingiva and synovial tissue

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SUMMARY

Using modified ELISA and spot-ELISA, which permit the parallel determination of heavy chain subclass and the presence of covalently linked J chain, we analysed IgA found in cell culture supernatants or secreted by individual cells from peripheral blood, spleen, bone marrow, gingiva and synovial tissue, with respect to its polymeric or monomeric IgA form (pIgA, mIgA) and IgA1 or IgA2 subclass. The ELISA for determination of J chain in tissue culture supernatants was specific and highly sensitive (detection limit in pg). The results demonstrated that IgA1-producing cells predominated in the tissues examined, and that J chain could be detected in association with the majority of IgA1 and IgA2 secreted by individual cells. With respect to the frequency of cells secreting polymeric, J chain-containing IgA, only 20–30% of cells from the bone marrow were engaged in the synthesis of pIgA. In other tissues the frequency of cells secreting pIgA1 and pIgA2 was considerably higher. Peripheral blood mononuclear cells secreting pIgA2 were easily inducible during stimulation with T cell-dependent pokeweed mitogen, whereas Epstein–Barr virus-transformed cells secreted preferentially mIgA1. When the frequencies of pIgA-, pIgA1- or pIgA2-secreting cells (determined by spot-ELISA technique) from different tissues were correlated with the proportion of pIgA to mIgA (and IgA subclasses) secreted in tissue culture supernatants, data obtained suggest that many individual IgA-producing cells could be engaged in simultaneous secretion of mIgA and pIgA.

Keywords IgA1 IgA2 polymeric IgA monomeric IgA IgA-secreting cells

INTRODUCTION

Two subclasses of human IgA have been identified on the basis of different antigenic determinants and differences in primary amino acid as well as carbohydrate structures (reviews by Mestecky & Russell, 1986; Mestecky *et al.*, 1989). IgA of both subclasses can be found in serum and in secretions although their respective proportions vary (Mestecky & Russell, 1986). Independently of the subclass, IgA molecules may be found as monomers or as covalently linked polymers containing J chain (Mestecky & McGhee, 1987; Mestecky *et al.*, 1989). Typically, IgA molecules found in serum are predominantly monomers of the IgA1 subclass. Although IgA1 is still the dominant subclass in all secretions examined, the percentage of IgA2 is increased in these fluids compared with serum. Moreover, most of the IgA found at secretory sites is in the polymeric conformation (Delacroix *et al.*, 1982).

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By means of molecular sieve chromatography, SDS–PAGE, sucrose density gradient ultracentrifugation, and immunofluorescence techniques, it has been demonstrated that most intracellular IgA occurs in monomeric form (mIgA) even in cells that secrete predominantly polymeric IgA (pIgA) (Parkhouse, 1971; Bargellesi, Perlman & Scharff, 1972; Kutteh, Prince & Mestecky, 1982; Moldoveanu, Egan & Mestecky, 1984). In view of these findings, it was proposed that polymerization occurs shortly before or at the time of secretion of the assembled IgA from the cells (Parkhouse, 1971; Bargellesi *et al.*, 1972; Moldoveanu *et al.*, 1984). Sequential analyses of IgA in the supernatant of peripheral blood mononuclear cells (PBMC) stimulated by pokeweed mitogen (PWM) revealed that the proportion of pIgA increased with the time of culture, and that polymers represented the prevalent form of secreted IgA from day 5 of *in vitro* culture (Kutteh *et al.*, 1980; 1982; Moldoveanu *et al.*, 1984). Although pIgA has been detected in supernatants from cultured lymphoid cells originating from various organs or cell lines (review by Koshland, 1985), it has not been established conclusively whether there are distinct populations of cells

secreting exclusively pIgA or mIgA, or whether one cell has the ability to secret both immunoglobulin forms simultaneously. Furthermore, Conley & Koopman (1982) have indicated that although PWM stimulation induces PBMC to differentiate into lymphoblasts containing equal numbers of cytoplasmic IgA1$^+$ or IgA2$^+$ cells, only IgA1 molecules were detected in the supernatants.

Using a technique which permits the determination of immunoglobulin secretion at the single cell level (Czerkinsky *et al.*, 1983), we attempted to extend our previous studies (Kutteh *et al.*, 1980, 1982; Moldoveanu *et al.*, 1984). The spot-ELISA assay was modified to permit analysis of frequencies of IgA1- and IgA2-secreting lymphocytes in unstimulated and mitogen-stimulated PBMC, Epstein–Barr virus (EBV) transformed cells, and in unstimulated cells from spleen, bone marrow, inflamed gingivae and synovial tissue. Furthermore, this new method allowed quantification of IgA subclasses with respect to J chain content (i.e. polymerization) at the single cell level.

MATERIALS AND METHODS

Isolation of cells and culture conditions

Human PBMC were isolated from healthy adult volunteers by centrifugation on a Ficoll–Hypaque (Sigma Chemical Company, St Louis, MO) density gradient. The cells were either used directly or resuspended at a concentration of 1×10^6/ml in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (FCS) (GIBCO), penicillin (100 U/ml), streptomycin (100 μg/ml) and 2·0 mM L-glutamine (GIBCO) (complete medium). The PBMC were stimulated with PWM (GIBCO) at a concentration 10 μl/ml and cultured at 37°C in a moist atmosphere containing 5% CO_2 in air. After a culture period varying from 2 to 7 days, the viable cells, obtained by another centrifugation on a Ficoll–Hypaque gradient, were assayed by the spot-ELISA assay. The cell supernatants were frozen at −20°C and analysed simultaneously by ELISA, in order to minimize interassay variation.

EBV-transformed cells were obtained by infecting Ficoll–Hypaque-isolated human PBMC or enriched B cells (obtained by removing cells rosetting with aminoethyl-isothiouronium bromide-treated sheep erythrocytes (Pellegrino *et al.*, 1975)), with medium from the EBV-producing B95–8 marmoset cell line (American Type Culture Collection, Rockville, MD). IgA1 and IgA2 clones established from EBV-transformed cells, isolated from human appendix (Miyawaki *et al.*, 1988) were a gift from Dr Max Cooper, University of Alabama at Birmingham.

Spleen tissues obtained from patients undergoing splenectomy due to idiopathic thrombocytopenic purpura were passed through a nylon mesh, and mononuclear cells were isolated on a Ficoll–Hypaque density gradient.

Bone marrow samples obtained from the iliac crest were treated with a lysing reagent (Ortho Diagnostics Systems, Raritan, NJ) and the mononuclear cells were washed and counted.

Synovial tissues from patients with rheumatoid arthritis, and gingival tissues from patients with adult periodontal disease, were obtained during synovectomy or gingivectomy, respectively. A modification of a previously described method for the preparation of single cell suspensions was utilized to obtain dissociated synovial and gingival cells (Frangakis *et al.*, 1982; Ogawa *et al.*, 1989). Briefly, the freshly obtained tissues were cut into 1-mm^3 fragments, washed extensively in cold Dulbecco's phosphate-buffered saline (PBS) and placed in Joklik's modified minimal essential medium (GIBCO) containing 1 mg/ml Dispase (grade 2, Boehringer Mannheim Biochemicals, Indianapolis, IN) at 37°C in a tissue culture flask, with constant agitation (1 g of tissue per 10 ml of enzyme solution). After 30 min, medium containing dissociated cells was removed by aspiration and was added into an equivalent volume of RPMI 1640 (GIBCO) containing 5% FCS. Fresh, pre-warmed enzyme solution was immediately added to the partially digested tissues, and the extraction procedure was repeated three to five times. Cell suspensions were then washed three to four times in complete medium. Cell viability, assessed by trypan blue dye exclusion, always exceeded 90%. Single cell suspensions were immediately assayed for IgA secretion by the spot-ELISA assay.

Enumeration of mIgA- and pIgA-secreting cells of a given subclass

The enumeration of frequencies of IgA-secreting cells of a given subclass was performed by spot-ELISA (Czerkinsky *et al.*, 1983). Briefly, shallow wells (2 cm in diameter) on lids from 24-well tissue culture clusters (Costar, Cambridge, MA) were coated overnight at room temperature with an IgG fraction of mouse monoclonal antibodies specific for human IgA1 or IgA2 (Crago *et al.*, 1984; Kett *et al.*, 1986). After blocking with PBS–5% FCS, for 1–2 h at room temperature, 100 μl of cell suspensions containing 10^3–10^5 mononuclear cells were added to each well and incubated for 3–4 h at 37°C. The cell layer was then removed and the dishes vigorously washed with PBS prior to the addition of biotin-conjugated F(ab′)$_2$ fragments of affinity-purified goat anti-human IgA (Tago, Burlingame, CA) diluted 1/750 in PBS–Tween 20 containing 1% FCS. After overnight incubation at room temperature, the unreacted biotinylated antibodies were removed and Vectastain® ABC-AP (alkaline phosphatase) complexes (Vector Laboratories, Burlinghame, CA) were added for 1 h. After another wash with PBS–Tween 20, the enzyme substrate (5-bromo-4-chloro-3-indolyl phosphate, 2·3 mmol/*l* in 2-amino-2-methyl-1-propranol buffer with magnesium ions, pH 10·2; Sigma) was added, and after approximately 1 h of incubation at room temperature the blue spots were counted.

In order to determine the presence of J chain in pIgA secreted by single cells, an approach similar to that described above was taken. However, after the cell incubation stage and wash, the wells were immersed in 3% paraformaldehyde for 20 min, washed briefly, and exposed to acidified 5 M urea, pH 2·5 for 30 min. This procedure, described originally by Brandtzaeg & Berdal (1975) for immunohistochemical purposes, partially denatures IgA and allows subsequent detection of J chain. After acid–urea exposure, the wells were incubated with PBS–10% FCS for 15 min, in order to block all the non-specifically reactive sites, and then incubated overnight at room temperature with rabbit anti-J chain antibody produced and characterized in our laboratory (Kutteh *et al.*, 1983), and, for this assay, adsorbed on mouse IgG (working dilution 1/750 in PBS–Tween–1% FCS). After another wash, biotinylated affinity-purified goat anti-rabbit antibody (Sigma), adsorbed against human immunoglobulins and diluted 1/1000 in PBS–Tween, was applied for 2 h at room temperature. The subsequent steps, involving washing and incubation with ABC-AP and enzyme-substrate solution,

were performed in exactly the same way as described above for the detection of IgA subclasses. Samples were always tested in duplicate using at least two different cell concentrations.

ELISA for determination of total and polymeric IgA1 and IgA2 in culture supernatants

Serial dilutions of mIgA1 (Pet), pIgA1 (Git), mIgA2 (Bul), and pIgA2 (Fel) purified myeloma protein standards (as appropriate for the assay in hand), as well as cell culture supernatants, all in PBS–10% FCS, were incubated overnight on 96-well polystyrene microtitre plates (Dynatech Laboratories, Alexandria, VA) coated and blocked in an identical way to the spot-ELISA dishes. Secondary antibody incubations, as well as paraformaldehyde and acid–urea treatment were also performed as in the respective spot-ELISA described above. Comparable results were obtained when 0·25% glutaraldehyde was used as fixative instead of paraformaldehyde. However, instead of ABC-AP, avidin–horseradish peroxidase (HRP, Sigma) (0·5 μg/ml in PBS–Tween) was used, followed by peroxidase substrate 2·2 azino-bis (3–ethyl-benzthiazoline-6-sulfonic acid) (Sigma) in citrate buffer, pH 4·2, containing 0·0075% H_2O_2. The absorbance was measured in a Titertek Multiscan photometer (Flow Labs, McLean, VA) at 414 nm. All samples were run in duplicate. The optical density (OD) values were converted to ng/ml using calibration curves based on the OD obtained from serial dilutions of the respective highly purified IgA myeloma proteins. The calibration curves were constructed using a computer program based on the weighted logit–log model (Russell *et al.*, 1986), on a Macintosh SE computer (Apple Computer, Cupertino, CA).

Radioimmunoassay for IgA and its ability to bind secretory component (SC)

Solid-phase radioimmunoassay was performed as previously described (Moldoveanu *et al.*, 1984), using polystyrene strips coated with anti-IgA, and developed with radioiodinated anti-IgA or SC. Purified myeloma IgA proteins were used as standards.

RESULTS

Sensitivity and specificity of the detection of J chain in pIgA1 and pIgA2

To establish the specificity and sensitivity of the detection of J chain in pIgA, ELISA wells coated with anti-human IgA1 or anti-IgA2 were incubated with purified IgA myeloma proteins of known subclass and J chain content. The fixation with paraformaldehyde, denaturation with acid–urea, and all the subsequent developing steps were applied as described in Materials and Methods, according to earlier pilot experiments. The results (Fig. 1) show that only pIgA but not mIgA myeloma proteins reacted with anti-J chain reagent. This assay system detected J chain in pIgA preparations containing less than 1 ng of protein/well. When the known content of J chain in pIgA is considered (by using dilutions of purified dimeric IgA), the assay is able to detect this polypeptide at a concentration of approximately 50 pg/well.

Similarly, when the spot-ELISA was applied to detect J chain in mIgA1 and mIgA2 secreted by clones derived from EBV-transformed cells, no J chain spots were detected despite high frequencies of subclass-specific IgA-secreting cells. These

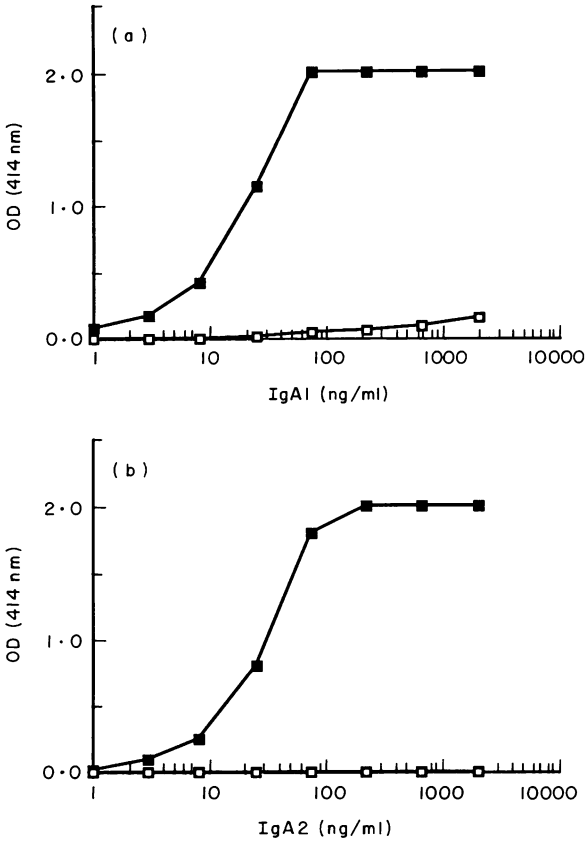

Fig. 1. Detection of J chain in purified IgA1 (a), and IgA2 (b) by ELISA using monomeric (□) and polymeric (■) IgA myeloma proteins (a, Pet and Git; b, Bul and Fel).

results indicated that the J chain reagent did not cross-react with either light or α chains.

Specificity of the anti-human IgA1 and anti-IgA2 monoclonal antibodies was confirmed by spot-ELISA in which the same clones that secreted exclusively IgA1 or IgA2 were incubated on plates coated with anti-IgA1 or anti-IgA2. After the incubation period, the plates were developed with a polyclonal anti-human IgA antibody. No cross-reactivity between IgA subclass antibodies was observed in this assay. As an additional control, PBMC from an IgA-deficient subject were cultured *in vitro* for 6 days in the presence of PWM. Despite a high frequency of IgM- or IgG-secreting cells only four pIgA1- and two pIgA2-secreting cells/10^6 PBMC were detected. This indicated that monoclonal anti-IgA1 and anti-IgA2 coating antibodies did not react with μ, γ or light chains. Incubation of the PBMC with the protein synthesis inhibitor, cycloheximide (50 μg/ml), for 2 h before and during plating reduced the number of IgA1, IgA2 and pIgA spots by approximately 90%

Secretion of pIgA1 and pIgA2 by lymphocytes from various tissues

Mononuclear cells from peripheral blood ($n=17$), spleen ($n=4$), bone marrow ($n=3$), gingiva ($n=3$) and synovial tissue ($n=3$) were analysed with respect to the frequency of IgA1- and IgA2-secreting cells, as well as the numbers of cells secreting pIgA1 or pIgA2. In all tissues examined there was a predominance of cells secreting IgA1 antibodies (Fig. 2). However, while

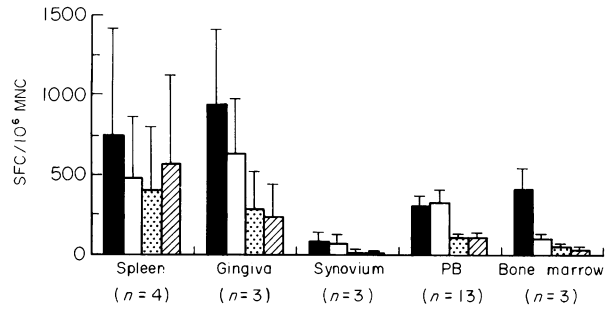

Fig. 2. Spontaneous secretion of total and polymeric IgA1 and IgA2 by mononuclear cells (MNC) derived from various tissues. Results are expressed as mean number (±s.e.m.) of spot-forming cells (SFC) per 10^6 MNC. ■, IgA1; □, pIgA1; ▨, IgA2; ▨, pIgA2. PB, peripheral blood.

Table 1. Percentage of J chain-positive cells in unstimulated PBMC and bone marrow*

	IgA	IgA1	IgA2
PBMC			
Donor A	76	84	63
Donor B	95	75	80
Bone marrow			
Donor A	29	24	51·5
Donor B	28	19·5	73·5

* Expressed as percentage of J chain-positive cells per total number of IgA^+, $IgA1^+$, or $IgA2^+$ cells.

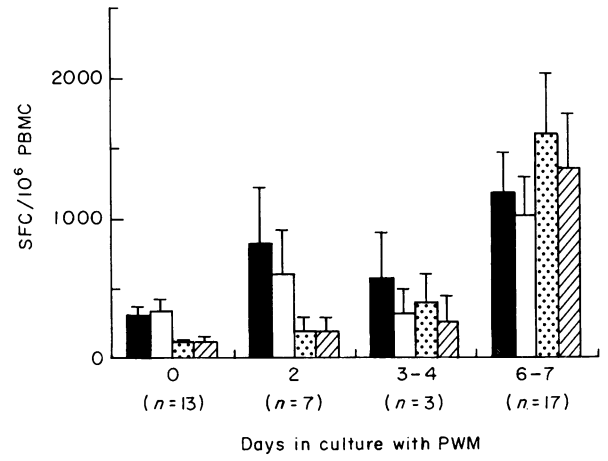

Fig. 3. Kinetics of secretion of total and polymeric IgA1 and IgA2 by peripheral blood mononuclear cells (PBMC). Results are expressed as mean number (±s.e.m.) of spot-forming cells (SFC) per 10^6 PBMC. ■, IgA1; □, pIgA1; ▨, IgA2; ▨, pIgA2. PWM, pokeweed mitogen.

the ratio of IgA1- to IgA2-secreting cells in synovium and bone marrow was 7·5 and 7·7, the ratio for spleen, gingival cells, and PBMC was 1·9, 2·9, and 3·3, respectively. The frequency of IgA-secreting cells varied considerably within as well as between different tissues. Interestingly, in all tissues studied, the IgA1

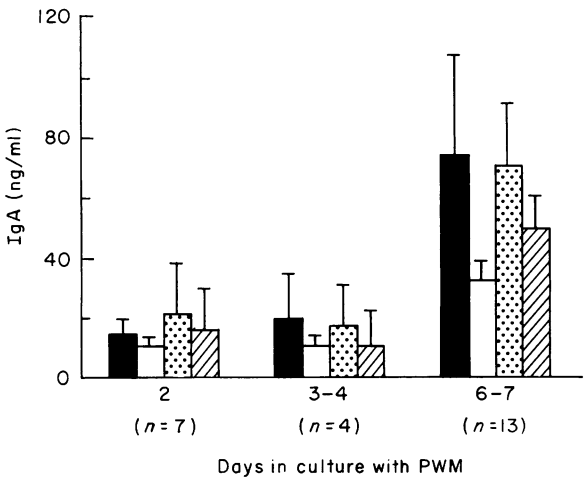

Fig. 4. Kinetics of the secretion of various forms of IgA into culture supernatant by peripheral blood mononuclear cells (PBMC) stimulated with pokeweed mitogen (PWM). Results are expressed as mean (±s.e.m.) of total and polymeric IgA1 and IgA2: ■, IgA1; □, pIgA1; ▨, IgA2; ▨, pIgA2.

and IgA2 secreted by individual cells were also positive for J chain in the ELISPOT assay (Fig. 2). Thus, the ratio of all IgA1-secreting cells to J chain-containing IgA1-secreting cells in PBMC, synovium, and gingiva was < 1·7, but in bone marrow the ratio was 4·2. Similar, although not as pronounced ratios were observed between the frequency of all IgA2 spot-forming cells and cells secreting pIgA2 (< 1·5 for PBMC, spleen, gingiva, and synovium, but 2·0 for bone marrow). The comparative frequencies of pIgA-, pIgA1- or pIgA2-secreting cells from two subjects as determined by the presence of J chain in the ELISPOT assay, are presented in Table 1. The data indicate that the IgA secreted by PBMC contained mostly pIgA, while in the bone marrow the number of spots that could be developed with anti-J chain and therefore representing pIgA production was about 28%. When analysed according to the distribution of pIgA1- or pIgA2-secreting cells, the frequency of pIgA2-secreting cells predominated in cells from bone marrow, although the absolute number of IgA2-secreting cells was higher in PBMC (Crago *et al.*, 1984). These results are in agreement with previously reported data concerning the numbers of J chain-positive cells disclosed by immunofluorescence (Brandtzaeg, 1976; Mestecky *et al.*, 1977).

Effect of mitogenic stimulation on secretion of pIgA1 and pIgA2

Cultures of PWM-stimulated PBMC were used to study kinetic changes in the secretion of pIgA1 and pIgA2. Although increases in numbers of IgA1- and IgA2-secreting cells were detectable as early as day 2 after *in vitro* stimulation, a more pronounced increase was seen on days 6 and 7 (Fig. 3). The ratio of IgA1- to IgA2-secreting cells was changed from 2·9 at day 0 to 0·7 after 1 week of incubation with PWM. During early and late periods of PWM-stimulation, the majority of cells produced pIgA1 and pIgA2 molecules (Fig. 3). Similar results, indicating time-dependent increases of active secretion of both pIgA1 and pIgA2, were observed in the cell culture supernatants as demonstrated by ELISA (Fig. 4). To reconcile the apparent discrepancy between the relatively high numbers of pIgA-

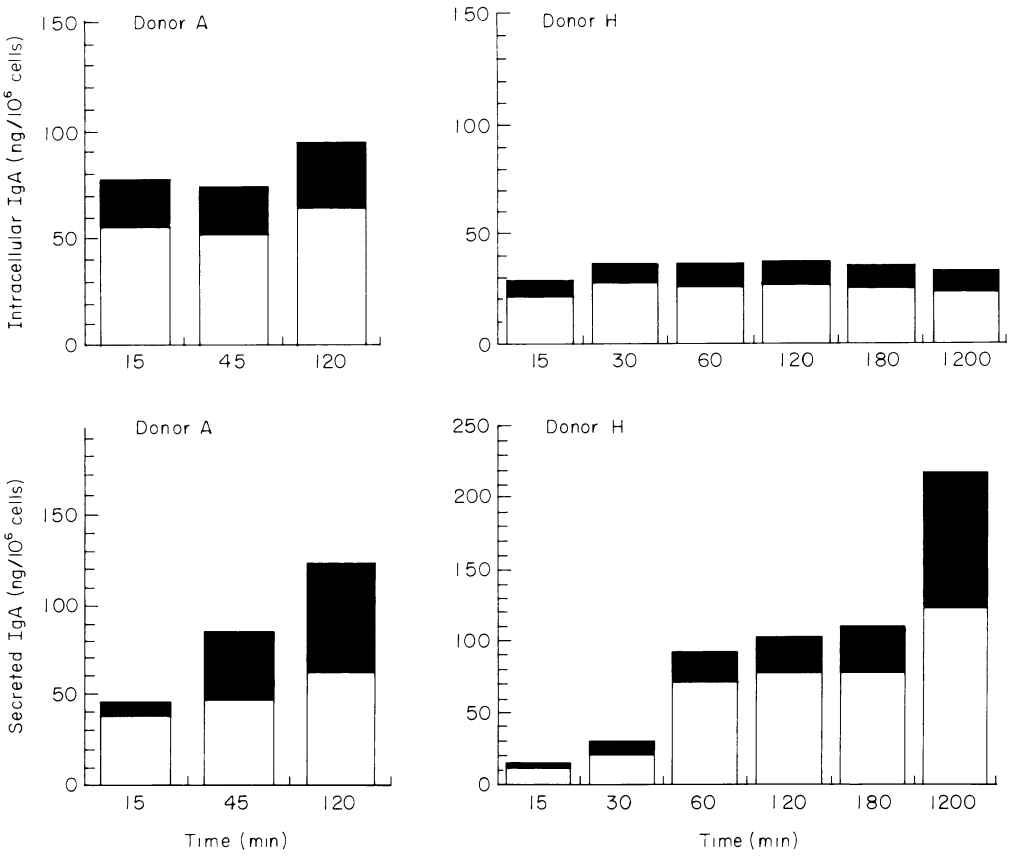

Fig. 5. Intracellular and secreted monomeric and polymeric IgA in peripheral blood mononuclear cells (PMBC) of two individuals (donor A and donor H). After 7 days culture in the presence of pokeweed mitogen (PWM), the cells were harvested, washed and divided into equal aliquots which were further incubated. At selected time intervals, the amounts of intracellular and secreted total and SC-binding IgA were measured in the cell lysates and in the corresponding supernatants. ■, IgA-binding SC; □, monomeric IgA.

Table 2. Spot-ELISA of IgA subclass and associated J chain secreted by EBV-transformed cells

	SFC/10^6 cells		J chain-positive cells (%)*
	IgA1	IgA2	
LTA-12 (α1, λ)	10350	0	1·5
LTA-13 (α1, κ)	22500	0	0·0
LTA-3 (α2, λ)	0	9000	2·8
LTA-15 (α2, κ)	0	950	15·8
LTA-17 (α2, κ)	0	1450	6·9
Donor C	6000	180	43·3
Donor D	300	0	0·0
Donor E	490	0	0·0
Donor A	8200	0	23·0
Donor F	2400	0	44·4
Donor G	120	245	0·0

* Expressed as percentage of J chain-positive spots per total number of IgA1- or IgA2-secreting cells.

SFC, spot-forming cells.

secreting cells as determined by spot-ELISA and the relatively low proportion of pIgA in the culture supernatants, one may postulate that pIgA and mIgA differ significantly in their secretion rates in favour of monomers. Because this point cannot at present be experimentally approached at the single cell level, we compared the proportion of pIgA-to-mIgA in lysed PBMC and corresponding culture supernatants at various time intervals after a 7-day culture in the presence of PWM. As an alternative method, we used the ability of SC to bind to pIgA. As seen in Fig. 5, the amount of total intracellular IgA (per 10^6 cells) as well as the proportion of mIgA to pIgA remained constant, with a pronounced preponderance of monomers, as reported earlier (Kutteh *et al.*, 1982; Moldoveanu *et al.*, 1984). In contrast, the total amount of IgA secreted into the culture media increased with time; the proportion of pIgA to total IgA also increased. These results also indicate that both molecular forms are secreted and there is no obvious preference for the secretion of mIgA. When EBV-transformed cells were studied by spot-ELISA high numbers of IgA1 spot-forming cells were detected (Table 2). J chain-containing IgA was found in some, but not all cell lines, and not in all cells within the same cell line.

DISCUSSION

One of the unresolved problems relating to the human IgA

system concerns the cellular origin of monomeric and polymeric forms of this immunoglobulin. The tissue distribution of mIgA- or pIgA-producing immunocytes has been determined by the examination of molecular forms of IgA in culture supernatants of tissue explants (Bull, Bienenstock & Tomasi, 1971; Buxbaum *et al.*, 1974; Kutteh *et al.*, 1982; MacDermott *et al.*, 1983), or immunohistochemically by the presence of J chain which presumably mediates the polymerization process, or by the exquisite and well established ability of pIgA-secreting cells or of pIgA in solution to bind SC (Brandtzaeg, 1973; Radl, *et al.*, 1974; Crago & Mestecky, 1979; Brandtzaeg, 1985; Moro *et al.*, 1990). These studies have indicated that IgA-producing cells in bone marrow secrete predominantly monomers (Kutteh *et al.*, 1982; MacDermott *et al.*, 1983), while in the gut both forms are produced, with a marked predominance of pIgA (Brandtzaeg, 1973; Buxbaum *et al.*, 1974; Kutteh *et al.*, 1982; MacDermott *et al.*, 1983; Brandtzaeg, 1985), as revealed by analysis of gut cell culture supernatants, gut perfusates and immunofluorescence. In other tissues, such as lymph nodes or spleen, IgA is present in both forms in variable proportions (Kutteh *et al.*, 1982). However, it has not been established convincingly whether there are two exclusive populations of cells secreting either one of these forms.

In order to address this question, we used, in addition to the SC-binding test, a novel, highly sensitive assay for quantification of pIgA of both subclasses secreted by single cells or released into culture supernatants. This method is based on the known ability of acid–urea to denature IgA partially, thus uncovering J chain epitopes which become available for subsequent immunochemical and immunohistochemical detection (Brandtzaeg, 1973; Brandtzaeg & Berdal, 1975). We were able to detect picogram amounts of IgA-bound J chain. The degree of sensitivity may be augmented even more by substituting avidin–HRP in ELISA with commercially available preformed avidin–biotin–HRP or avidin–biotin–AP complexes. We have recently shown that levels of antigen-specific pIgA antibodies, as determined by ELISA, correlated well with the expected proportion of pIgA observed by HPLC (Lue, Tarkowski & Mestecky, 1988).

Our results, based on the technique described above, indicate that a high proportion of cells, irrespective of their origin, have the capacity to secrete pIgA. The sensitivity of the assay used for the detection of pIgA may be higher than that for the detection of IgA1- or IgA2-secreting cells as seen in some samples (Fig. 2), possibly due to amplification arising from the use of a secondary (goat anti-rabbit) antibody, and, furthermore, pIgA standards were used to quantify secreted IgA. pIgA-secreting cells were detected in various tissues in surprisingly high numbers suggestive of predominant pIgA synthesis. Yet, when related to the forms of IgA released into culture supernatants (Kutteh *et al.*, 1980, 1982; MacDermott *et al.*, 1983; Moldoveanu *et al.*, 1984) (Figs 3–5), the data suggested that one IgA cell can apparently produce both pIgA and mIgA. For example, close to 99% of PWM-stimulated PBMC secreted pIgA (as detected by spot-ELISA), while their culture supernatants contained less than 45% of pIgA. Similarly, the proportion of bone marrow or spleen cells secreting pIgA was considerably higher than the very low proportion of pIgA in culture supernatants (Kutteh *et al.*, 1982; MacDermott *et al.*, 1983). These differences in the number of $pIgA^+$ spots and the proportion of pIgA-to-mIgA in culture supernatants are not due to the preferential secretion of monomers (Fig. 5). However, for technical reasons, we cannot estimate at the single cell level the relative proportion of pIgA *versus* mIgA secreted. It is probable that in our experiments, secreted pIgA may have constituted only a minute fraction of the total IgA secreted by a single cell. Comparative molecular and cellular analyses of the molecular forms of intracellular *versus* secreted IgA from PWM-stimulated PBMC and IgA-secreting cell lines (Kutteh *et al.*, 1983; Moldoveanu *et al.*, 1984; Moro *et al.*, 1990) revealed that the presence of intracellular J chain and the ability of fixed IgA-containing cells to bind SC does not necessarily indicate that such cells are engaged in exclusive synthesis of pIgA. Furthermore, cells secreting pIgA in the supernatant contained intracellular IgA predominantly in the mIgA form with a small amount of pIgA which was apparently responsible for the ability of such cells to display a positive SC-binding test and the presence of J chain. Thus, we infer that despite a relatively high proportion of $pIgA^+$ cells found by spot-ELISA, for example, in the bone marrow, the actual amount of polymers secreted is very small. The mechanisms involved in the regulation of the proportion of pIgA and mIgA secreted by cells from different tissues are at present unknown. The availability of intracellular J chain or other factors involved in polymerization (Koshland, 1985) may limit the assembly of polymers: the excess of intracellular mIgA which is not polymerized may be secreted in this form. Furthermore, it has been demonstrated that cells stimulated *in vitro* with various mitogens or *in vivo* with antigens introduced either by mucosal or systemic routes produce predominantly pIgA (Lue *et al.*, 1988). Sera of subjects recently exposed to a variety of viral infections or systemically immunized with vaccines, contained predominantly pIgA in the early phase of the specific IgA response (Akahonai *et al.*, 1985; Brown *et al.*, 1985; Negro-Ponzi *et al.*, 1985; Hikata *et al.*, 1986; Mascart-Lemone *et al.*, 1987a, 1987b, 1988; Lue *et al.*, 1988; Mestecky *et al.*, 1989). Also, lymphoid cells from chronically inflamed tissues secrete high proportions of pIgA (Ogawa *et al.*, 1989). These results suggest that in early stages of stimulation, specific antibodies are represented predominantly by IgA polymers.

If we accept that many human lymphoid tissues can produce pIgA, we must try and explain the relatively low proportion of pIgA in human serum. First, it should be re-emphasized that most of the IgA in human serum is derived from the bone marrow (Mestecky *et al.*, 1977; Kutteh *et al.*, 1982; MacDermott *et al.*, 1983; Mestecky & McGhee, 1987). Second, the pIgA is cleared from the circulation at a faster rate than mIgA (Delacroix *et al.*, 1983). Although human hepatocytes, unlike those of rats, mice, and rabbits, do not apparently express SC, there is a preference for the binding of pIgA by human liver (Tomana, Kulhavy & Mestecky, 1988). Furthermore, in individuals with impaired liver function, part of the increase in the pIgA fraction in serum has been ascribed to retarded elimination of pIgA (Delacroix *et al.*, 1983).

Using the assay described here, we were able to detect cells secreting either IgA1 or IgA2 in samples of bone marrow, spleen, synovial tissue, and gingiva, and in PWM-stimulated and non-stimulated PBMC. In this respect, identical results were reported by Islam *et al.* (1990), using PWM- or EBV-stimulated PBMC and IgA subclass-specific monoclonal antibodies from a different source. These results differ from an earlier report (Conley & Koopman, 1982), in which $IgA2^+$ cells

were easily detectable by immunofluorescence but did not secrete their product into culture supernatants. We have established that the IgA2 spots do not result from IgA2 released from dying cells (spot formation was inhibited by pretreatment of the cells with cycloheximide), nor from cross-reactivity of the monoclonal antibodies used in this assay with $\alpha 1$, γ, or μ heavy or light chains. Identical conclusions were reached by Islam *et al.* (1990). Furthermore, the sum of IgA1- and IgA2-secreting cells (present study) or IgA1- and IgA2-containing cells enumerated with the use of the same fluorochrome-labelled monoclonal antibodies (Crago *et al.*, 1984; Kett *et al.*, 1986) was in close agreement. Thus, we can speculate that the reason for this apparent discrepancy in the ability of IgA2 cells to secrete their product could be explained by the use of different monoclonal reagents.

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