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Differential G-protein expression during B- and T-cell development

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SUMMARY

The molecular mechanisms underlying B- and T-cell development are, as yet, poorly understood. However, as G proteins regulate a diverse range of biological responses including growth, proliferation and differentiation, we have investigated differential expression of G proteins during B- and T-cell development with the aim of identifying key signals involved in lymphocyte maturation. Differential expression of $\beta_{1/2}$ and α-subunits of the Gs-, i- and q-families was found throughout lymphoid development. Most strikingly, $G\alpha_{i1}$ and $G\alpha_{11}$ were very weakly, or not expressed in pre-, immature and mature B cells, thymocytes or mature T cells, but strongly induced in mature B-lymphoblastoid cell lines, some of which have been used as models of germinal centre B cells, suggesting that expression of these G proteins may correlate with the later stages of B-cell development. In contrast, $G\alpha_{16}$ expression was highest in T cells and pre-B cells and progressively declined with B-cell maturation. These findings suggest that G proteins, and the signals they regulate, such as ion channels and/or adenylate cyclase ($G\alpha_{s/i}$) and phospholipase C ($G\beta\gamma$ and $G\alpha_{11/16}$) are differentially regulated in lymphoid cells in a maturation- and lineage-dependent manner.

INTRODUCTION

The development of haemopoietic pluripotential stem cells into antibody-secreting B cells or helper or cytotoxic T cells is a complex process, integrating signals generated by a number of immunoregulatory receptors on several cell types, often in specialized environments. The molecular mechanisms underlying these developmental processes are, as yet, poorly understood. However, guanine nucleotide-binding proteins (G proteins) constitute a group of regulatory molecules which have been shown to be involved in the regulation of a diverse range of biological processes such as intracellular trafficking, cell movement, growth, proliferation and differentiation (reviewed in refs. 1–3). In this manuscript we have therefore investigated differential expression of G proteins during B- and T-cell development with the aim of identifying the key signalling pathways involved in lymphocyte maturation.

The G-protein superfamily predominantly comprises two major subfamilies, the small, monomeric *ras*-like G proteins and the heterotrimeric G proteins. Heterotrimeric G proteins regulate the transduction of transmembrane signals from cell surface receptors to a variety of intracellular effectors, such as adenylate cyclase and phospholipase C (PLC).[1–6] These G proteins consist of three distinct classes of subunits, α (39000–46000 MW), β (37000 MW) and γ (8000 MW) and in general, effector specificity is conferred by the α-subunit, which contains the GTP binding site and an intrinsic GTPase activity.[1–3] However, it is now widely accepted that $\beta\gamma$ complexes can also directly regulate effectors such as phospholipase A_2, PLC-β isoforms, adenylate cyclase and ion channels in mammalian systems and in addition, cellular responses such as mating factor receptor pathways in yeast.[4–9]

Several distinct subfamilies of heterotrimeric G proteins have been isolated and cloned: at least seventeen different α-subunit genes have been identified in mammals and these have been divided into four major subfamilies: G_s, G_i, G_q and G_{12}.[1–4] Moreover, cDNA clones encoding at least four related, but distinct, β-subunits and six γ-subunits have been identified.[10–14] The G_s family contains G_s and G_{olf}: these α-subunits stimulate adenylate cyclase, and can be irreversibly activated by ADP-ribosylation by cholera toxin.[1–4] The G_i family contains G_i, G_o and G_z subunits, which can inhibit adenylate cyclase and modulate potassium and calcium channels.[1–4,7,15] In addition, $\beta\gamma$-subunits resulting from activation of pertussis toxin-sensitive G_i-like G proteins ($G_i\alpha$ and G_{oa}) have been shown to regulate β-isoforms of PLC.[1–4,6] Interestingly, the α-subunits of the G_q subfamily (G_q, G_{11}, G_{14}, G_{15} and G_{16}) have also been shown to activate the β-isoforms of PLC.[2–6,16] Although the G_{12} subfamily, which contains G_{12} and G_{13}, is less well characterised, roles for these G proteins have been implicated in the regulation of sodium ion–hydrogen ion exchange (G_{13}) and eicosanoid production (G_{12}).[17,18]

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Many of the G_α subunits have been shown to exhibit a rather ubiquitous pattern of expression in mammalian systems, at least at the mRNA level but it is also clear that certain α-subunits have a tissue-restricted profile of expression.[1–4] Moreover, there is evidence of differential expression of certain α-subunits during cellular ontogeny.[19–21] Indeed, $G_{15\alpha}$ and $G_{16\alpha}$ have been shown to be haemopoietic lineage-restricted G proteins: although $G_{15/16\alpha}$ appear to be expressed in some myeloid cells, such as the HL-60 promyelocytic cell line, induction of terminal differentiation of HL-60 cells into neutrophil-like cells has been shown to result in a 90% decrease in $G_{16\alpha}$ concomitant with a 160% increase in $G_{i2\alpha}$ expression.[1,21] In contrast, sustained expression of $G_{15/16\alpha}$ appears to correlate with differentiation to the lymphoid lineage.[1,21] Thus while loss of $G_{16\alpha}$ expression could perhaps signal a loss of 'stemness'/pluripotentiality from the myeloid precursor cells, the increase in $G_{i2\alpha}$ expression could correlate with commitment to a neutrophil phenotype. Likewise, sustained expression of $G_{16\alpha}$ following terminal differentiation appears to indicate commitment to a lymphoid lineage. Hence, differential expression of particular G proteins could play a role in haemopoietic lineage determination. We have therefore screened a panel of B and T cells of both primary tissue origin, and also from transformed cell lines 'frozen' at defined stages of development, for maturation stage-specific patterns in their complement of G-protein subunit expression and have found that there is differential expression of $\beta_{1/2}$- and α-subunits of the Gs-, i- and q-families throughout lymphoid development.

METHODS

B- and T-lymphocyte preparation and cell culture

Small, dense B (>90% sIg^+) and T (>90% $CD3^+$) cells were prepared from the spleens of 12 (mature) or four (immature) week old BALB/c mice as described previously.[22] The 4-week-old B cells were classified as 'immature' as they do not induce DNA synthesis in response to anti-immunoglobulin and are thus not functionally identical to B cells from adult mice.[22] Briefly, B cells were prepared by depleting T cells with anti-Thy-1 and complement, followed by Percoll density fractionation[22] and T cells were purified by depleting B cells (and adherent cells) by two cycles of adherence to plastic Petri dishes coated with goat anti-mouse immunoglobulin antibodies (Sigma, Poole, UK), followed by percoll density centrifugation.[22]

Thymocytes were isolated as described previously.[22] Briefly, thymic tissue was removed from 4-week-old BALB/c mice and pressed through steel gauze to obtain a single cell suspension. Mononuclear cells obtained by Ficoll–paque density centrifugation were then subjected to differential Percoll density gradient centrifugation.[22]

Murine thymocytes and lymphocytes (10^6 cells/assay) were stained with Quantum Red Streptavidin (Sigma)-biotinylated anti-CD3 antibody (1 μg/ml), fluorescein isothiocyanate (FITC)-labelled anti-CD4 antibody (1 μg/ml), phycoerythrin (PE)-labelled anti-CD8α antibody (1 μg/ml) (all obtained from Pharmingen, Cambridge Bioscience, Cambridge, UK) and FITC-labelled anti-immunoglobulin antibody (1 μg/ml) (Sigma) for analysis of thymocyte and lymphocyte populations, respectively, by flow cytometry (Becton-Dickinson Facscan, Sunnyvale, CA). B cells were stained for surface immunoglobulin M and D (sIgMsIgD) expression with a Quantum Red Streptavidin (Sigma)-biotinylated rat anti-mouse anti-μ monoclonal antibody (mAb), b.7.6 (1 μg/ml) and FITC-labelled anti-δ mAb (1 μg/ml), either mouse anti-Igh-5^a (AMS 28.11) or rat anti-mouse anti-δ (1.19). The WEHI-231 cells used in this study do not have detectable sIgD expression.

Human B and T cells were prepared from a single cell suspension derived from tonsils obtained from routine surgery as described previously.[22] Mononuclear cells were obtained by Ficoll–Hypaque centrifugation, washed three times and pelleted with AET–SRBC (sheep erythrocytes coated with 2-aminoethylisthiouronium bromide). Fetal calf serum (FCS; 1 ml) was layered on top of the pellet followed by a 30-min incubation on ice. The supernatant and pellet were centrifuged through Ficoll and non-rosetted cells (B cells) were collected from the interface and washed. T cells, which had been rosetted with SRBC, were obtained following lysis of SRBC by ammonium chloride. These B- and T-cell-enriched populations were centrifuged through discontinuous percoll gradients and high-density, quiescent B and T cells were harvested from the 1·09 g/ml–1·08 g/ml interface. Enriched B-cell (>95% $CD19^+$) and T-cell (>95% $CD3^+$) populations were analysed by flow cytometry using FITC-labelled anti-human CD19 (pan-B cell marker) and PE-labelled anti-human CD3 mAb from Pharmingen, Cambridge Bioscience, Cambridge, UK. B cells were stained for sIgMsIgD expression with a PE-conjugated goat anti-human IgM (μ-chain specific) antibody and a FITC-labelled goat anti-IgD (δ-chain specific) antibody from Sigma, Poole, UK.

The human pre-B-cell lines, 207, 697 and REH were a kind gift from Dr Robin Callard, Institute of Child Health, London UK, and all other cell lines were obtained from the European Collection of Cell Cultures (ECACC), Porton Down, UK. The human cell lines were maintained in RPMI-1640 medium, supplemented with 10% FCS, 2 mM L-glutamine, penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37° in a 5% (v/v) CO_2 atmosphere at 95% humidity: the murine cell lines were also grown under these conditions but with medium additionally supplemented with 2-mercaptoethanol (final concentration 50 μM).

G-protein antisera

The production and specificities of the rabbit polyclonal antibodies raised against peptides specific for individual G protein α- and β-subunits, and their linearity of reactivity, have been described previously.[23] Antiserum BN3 is raised against peptide 1–10 of β_1 (MSELDQLRQE); CS3 is α_s 385–394 (RMHLRQYELL); IM1 is α_o 22–35 (NLKEDGISAAKDVK); ON1 is α_o 2–17 (GCTLSAEERAALERSK); OC1 is the α_o C-terminal decapeptide (ANNLRGCGLY); SG1 is α_t 341–350 (KENLKDCGLF) and also recognizes $G\alpha_{i1/2}$; I1C is α_{i1}159–168 (LDRIAQPNYI); I3C/I3B is α_{i3} 345–354 (KNNLKECGLY); IQB is α_q 119–134 (EKVSAFENPYDAIKS); CQ1 is the common determinant of α_q 351–360 and α_{11} 350–359 (QLNLKEYNLV) and 13CB is the α_{13} C-terminal decapeptide (HDNLKQLMLQ). The anti-$G_{\alpha 16}$ antibody was a kind gift from Prof. P. Gierschik, Department Pharmacology, University of Ulm, Germany.

Western blot analysis of G-protein expression

Cells were washed twice in 50 mM Tris buffer pH 7·4 containing 150 mM NaCl (TBS) and then resuspended (at 10^8 cells/ml) in 50 mM Tris buffer, pH 7·4 containing 150 mM NaCl; 1% nonidet P.40 (NP-40); 0·25% sodium deoxycholate; 1 mM ethylene glycol-bis(β-aminoethyl ether) tetraacetic acid (EGTA); 1 mM phenyl methyl sulphonyl fluoride (PMSF); 1 μg/ml antipain, chymostatin, leupeptin and pepstatin A; 1 mM Na_3VO_4 and 1 mM NaF (RIPA buffer). Cells were lysed on ice for 15 min and then microfuged at 15 640 *g* for 30 min at 4°. The supernatants (cell lysates) were then transferred to fresh tubes and stored at −20°. Protein estimations of cell lysates were made using the Biorad Dc Protein Estimation Kit. A rat brain membrane preparation[23] was used as a positive control for the non-haemopoietic cell-restricted G proteins. Cell lysates [5×10^6 cells/lane (Fig. 1) or 100 μg/lane (results not shown)] or rat brain membranes (50 μg/lane) were electrophoresed on 7·5% polyacrylamide gels according to the method of Laemmli.[23] Loading buffer (50 mM Tris–HCl buffer, pH 8 containing 5 M urea, 0·17 M sodium dodecyl sulphate (SDS), 0·4 M dithiothreitol (DTT), 0.01% (w/v) Bromophenol blue) was added in equal volume to the samples. Following SDS–polyacrylamide gel electrophoresis (PAGE), proteins were semi-dry blotted using a Sartoblot apparatus according to the manufacturers instructions (Sartorius Ltd, Langmead Business Centre, Epsom, Surrey, UK) on to nitrocellulose membranes (Hybond-ECL, Amersham, Bucks, UK). Blots were blocked in 5% (w/v) gelatine and 10% (v/v) donkey serum in TBS (pH 7·6) containing 0·1% (v/v) Tween-20 (Tris-buffered saline, TBS–T) for 1 h at 37°. Blots were then incubated in anti-G protein antibodies diluted 1 : 1000 in 0·25% (w/v) gelatine, 3% (v/v) donkey serum in TBS–T overnight at 4°. Donkey anti-rabbit immunoglobulin–horseradish peroxidase (HRP) conjugate (Amersham) was used as secondary antibody (diluted 1 : 1000 in 3% (v/v) donkey serum in TBS–T), for 2 hr at room temperature. Blots were developed using an enhanced chemiluminescence system (ECL Detection Kit and Hyperfilm-ECL, Amersham). Equal protein loading and even electrotransfer were checked by Ponceau Red staining.

The data in Fig. 1 represent G-protein expression in lymphocytes based on lysates of cell equivalents (5×10^6/lane). However, many of these experiments were also performed on extracts of equivalent protein loading (100 μg/lane) with essentially similar results. The results presented in Table 1 are therefore pooled from several experiments utilizing independently prepared cell lysates of both cell equivalents and equal protein loadings.

RESULTS AND DISCUSSION

Affinity labelling of G proteins expressed in membrane fractions of murine splenic B cells with [α-^{32}P]GTP indicated the presence of putative G-protein α-subunits (MW 40 000–45 000) which potentially could be differentially expressed during B-cell development (results not shown). Targeting of Gs and Gi α-subunits by cholera or pertussis toxin-mediated ADP-ribosylation, confirmed that the bands identified by affinity labelling studies were indeed likely to be Gα-subunits and that Gs and Gi-like α-subunits are, as expected, expressed in B cells derived from mature murine spleens cells (results not shown). These results confirm previously published data showing Gα-subunit expression in pre-B, immature, (WEHI 231 immature B cell lymphoma) and mature, B cells.[24–26] Differential expression of G proteins during B-cell development was investigated by studies using a panel of antibodies specific for individual G_α-subunits.

The possibility of differential $G\alpha_s$-expression during lymphopoiesis was investigated by specific detection of a 45 000 MW protein by an antiserum, CS1 (Fig. 1a), which is raised against the C-terminal peptide of mammalian $G\alpha_s$. These studies suggested that although $G\alpha_s$ is generally quite highly expressed in B and T cells and a number of proliferating B- and T-cell lines (Fig. 1a), the human mature lymphoblastoid B-cell lines, Ramos (lane 2) and Daudi (lane 3), the murine immature B cell lymphoma WEHI 231 (lane 8) and the human pre-B cell line, 697 (Table 1 and results not shown) appeared to express little or no $G\alpha_s$. This lack of $G\alpha_s$ expression cannot be due to these cells sharing a common maturation stage as whilst WEHI-231 cells are commonly used as a model of immature B cells, Daudi ($CD38^+$) and Ramos are used as a model of germinal centre B cells.[27,28] However, these are all proliferating B-cell lines and as cAMP is generally considered to be an anti-proliferative signal for lymphocytes,[29] although there does not appear to be an absolute correlation between proliferation and decreased $G\alpha_s$ expression as evidenced, for instance, by the high $G\alpha_s$ expression in the proliferating pre-B-cell lines, REH and 207, the B-cell line, Cess and the human leukaemic T-cell line, Jurkat, these results may suggest that the processes leading to cellular activation and proliferation, or immortalization of certain lymphoblastoid cell lines, may involve down-regulation of $G\alpha_s$ expression and consequent potential cAMP generation. Similarly, whilst antisera specific for α-subunits of the G_i subfamily, G proteins which are likely to be involved in reducing basal cAMP signals, identified G_i-like proteins in all lymphoid cells examined (Fig. 1b–d), total $G\alpha_i$ (predominantly $G\alpha_{i2}$) expression was highest in proliferating lymphocyte cell lines, such as the pre-B-cell lines, REH (lane 6), Nalm 6 and 697 (Table 1 and results not shown), the lymphoblastoid cell lines Daudi, Ramos and Cess (lanes 2–4) used as model systems for follicular B cells[27,28,30] and the leukaemic T-cell line, Jurkat (lane 9). Indeed, our preliminary experiments, using a commercial pan-$G\alpha_i$ antibody (NEI-800; ref. 23), indicate that B cells activated *in vivo* (large, low-density splenic B cells) express more Gi-like α-subunits than small, dense resting B cells (results not shown). In contrast, total $G\alpha_i$ expression was lowest in quiescent splenic B and T cells and in lymphocytes, such as thymocytes and immature B cells (WEHI 231 cells and 4-week-old splenic B cells), which respond to cross-linking of their antigen receptors by anergy and/or programmed cell death by apoptosis.[22] These are cellular responses in which cAMP elevation has been implicated as playing an important role.[31,32]

Interestingly, whilst no $G\alpha_{i1}$ expression could be detected in pre-B-cell lines, immature or mature resting B or T cells (Fig. 1c), induction of $G\alpha_{i1}$ expression does appear to correlate with late differentiation of the B-cell lineage as it is expressed in human lymphoblastoid B-cell lines, commonly used as models of follicular/germinal centre cells.[27,28,30] Moreover, loss of $G\alpha_{i1}$ expression could perhaps differentiate between lymphoid (Fig. 1c) and myeloid precursors as there is weak expression in the monocytic cell line, HL-60 (Table 1 and results not shown; refs. 33, 34). Similarly, $G\alpha_{i3}$ which is

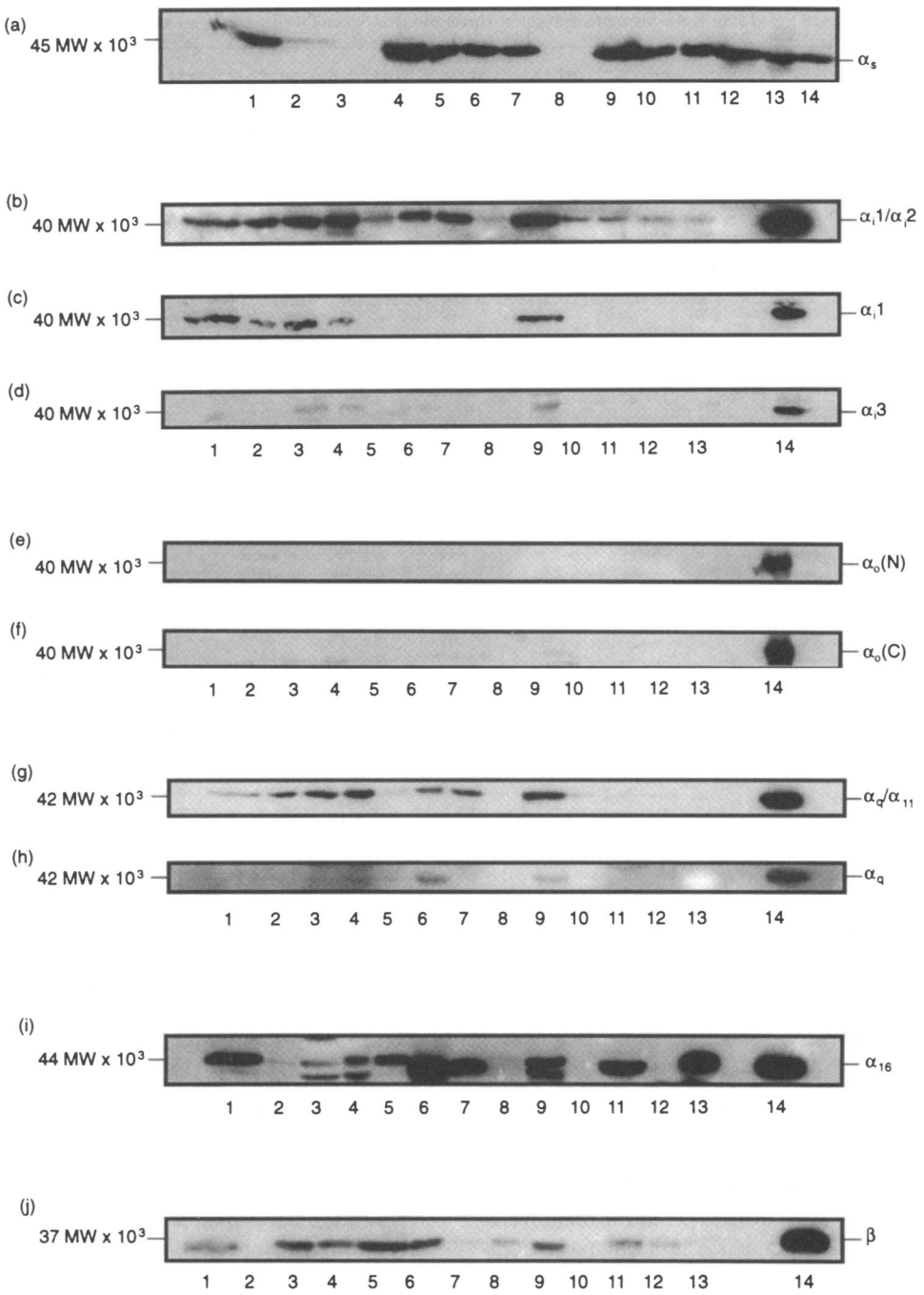

Figure 1. G-protein expression during B- and T-cell maturation. The cells (5×10^6/lane) investigated for G-protein expression (Gαs, o, i, q and 11 and $G\beta_{1/2}$) were: in panels, (a)–(h) and (j): the human B lymphoblastoid cell lines, Raji (lane 1), Ramos (lane 2), Daudi (lane 3), Cess (lane 4); the human pre-B cells, 207 (lane 5) and REH (lane 6); murine splenic B cells (lane 7); the murine immature B-cell lymphoma WEHI 231 (lane 8); the human leukaemic T-cell line Jurkat (lane 9); murine splenic T cells (lane 10); murine thymocytes (lane 11); murine 4-week-old splenic B cells (lane 12); the erythroleukaemia Clone 707 (lane 13) and rat membranes (lane 14). For immunodetection of $G\alpha_{16}$, (i), the cells tested were the human B lymphoblastoid cell lines, Raji (lane 1), Ramos (lane 2), Daudi (lane 3), Cess (lane 4); the human pre-B cells, 207 (lane 5) and REH (lane 6); human tonsil B cells (lane 7); the murine immature B-cell lymphoma WEHI 231 (lane 8); the human leukaemic T-cell line Jurkat (lane 9); murine splenic T cells (lane 10); human tonsil T cells (lane 11); murine 4-week-old splenic B cells (lane 12); the erythroleukaemia Clone 707 (lane 13) and the human monocytic cell line, HL-60 (lane 14).

generally considered to be ubiquitously expressed in mammalian tissues, and indeed, is highly expressed in the monocytic cell line, HL-60 (ref. 34 and results not shown), was not detected during murine lymphopoiesis (Fig. 1d), and only weakly detected in human pre-B cells and human B and T cells. These results may therefore suggest that $G\alpha_{i1/3}$ may play less prominent roles in (murine) lymphopoietic cell signalling than in myeloid cells and other tissues, a possibility consistent with

Table 1. G-protein expression throughout B- and T-cell development

	αs	αo	αi1	αi2	αi3	αq	α11	α13	α16	β1
Rat brain membranes	+ + +	+ + + +	+ +	+ + + +	+ +	+ + +	+ + + +	+	ND*	+ + +
Erythroleukaemia	+ + +	–	–	+	±	–	–	–	+ + +	+
Clone 707										
Monocytes -HL-60	+ +	–	+ +	+	+ +	+	+	+	+ + + +	+ +
B-cell lineage										
pre-B cells REH (cyt μ⁻)	+ + +	–	–	+ +	+	+	±	–	+ + + +	+ +
pre-B cells Nalm 6 (μ,δ⁻)	+ +	–	–	+ +	+	ND	±	–	+ + + +	+ +
pre-B cells 697 (μ,δ⁻)	±	–	–	+ +	+	ND	±	–	+ + + +	+ +
pre-B cells 207 (μ⁺ cyt κ)	+ + +	–	–	+	+	±	±	–	+ +	+ + +
WEHI-231 immature B cells (IgM⁺ IgD⁻)	±	–	–	+		±	–	–	–	+
4 week splenic B cells (80% IgM⁺ 26% IgD⁺)	+ + +	–	–	+	–	–	–	–	–	+
mature splenic B cells (IgM⁺ IgD⁺⁺)	+ + +	–	–	+ +	–	±	+	–	ND	±
mature tonsil B cells (IgM⁺ IgD⁺⁺)	+ +	–	ND	+	±	ND	ND	ND	+ + +	ND
Daudi B cells (IgM⁺ IgD⁻)	±	–	+	+ +	+	±	+ +	–	+	+ + +
Ramos B cells (IgM⁺ IgD⁻)	+	–	+ +	+ +	±	±	+ +	–	±	–
Cess B cells (IgM⁺ IgD⁻)	+ + + +	–	+	+ + +	+	+	+ + +	–	+ +	+ +
Raji B cells (IgM⁻ IgD⁻)	+ +	–	+ +	+	+	±	+	–	+ + +	+ + +
T-cell lineage										
thymocytes	+ +	–	–	+	–	–	–	–	ND	+ –
Jurkat T cells	+ + +	–	+ +	+ + +	±	–	+ +	–	+ + +	+ +
splenic T cells	+ +	–	–	+	–	–	–	–	–	–
tonsil T cells	+ +	–	ND	+	ND	ND	ND	ND	+ + +	ND

*ND, not determined; ±, barely detectable signal; +, weak signal; + +, moderate signal; + + +, strong signal; + + + + very strong signal.

the proposal that lymphoid cells appear to have a biased profile of Gα subunit expression as evidenced by the restriction of $G\alpha_{15/16}$ to haemopoietic, and particularly early B lineage, cells.[1,21,26,35] Moreover, antibodies specific for the $G\alpha_i$-like G_o α-subunits (OC1, ON1 and IM1), which strongly recognized a protein of approximate MW 40 000 kDa in rat brain membranes, did not identify $G\alpha_o$ expression in haemopoietic cells (Fig. 1e, f and results not shown), a finding entirely consistent with widely reported findings that Go proteins are restricted to neural and or endocrine tissue.[1,4]

Expression of the pertussis and cholera toxin-insensitive $G\alpha_q$ and $G\alpha_{12}$ subfamilies of α-subunits during lymphopoiesis was investigated by Western blot analysis using isoform-specific antisera (Fig. 1g–i): despite the fact that $G\alpha_{13}$ is generally considered to be one of the classes of α-subunits to be ubiquitously expressed at the mRNA level in mammalian systems,[1,4] it was not possible to detect this G protein in any of the haemopoietic cells tested, and it was only very weakly detected in rat brain membranes and HL-60 cells (results not shown). However, it has previously been reported that $G_{13\alpha}$ can only be detected in mammalian brain tissues at the mRNA level as the protein is not expressed in sufficient quantities to be detected by Western blotting[36] and thus, it cannot be ruled out that $G\alpha_{13}$ may also be expressed at very low levels in haemopoietic cells.

Blotting with an antiserum, IQB which is specific for $G\alpha_q$ suggested that lymphocytes only expresses $G\alpha_q$-like proteins at very low levels (Fig. 1h). Indeed, $G\alpha_q$ could only be convincingly detected in the human pre-B cell line, REH (lane 6), the human lymphoblastoid cell line, Cess (lane 4) and the human leukaemic T-cell line, Jurkat (lane 9). A similar, but very weak, pattern of expression was detected with antiserum 14CB, which recognizes epitopes common to $G\alpha_q$ and $G\alpha_{14}$ (results not shown) and was consistent with an earlier report that $G\alpha_{14}$ expression was restricted to stromal cells, early myeloid cells and progenitor B cells.[35] However, CQ, an antibody which is specific for the C-terminal region which is common to both $G\alpha_q$ and $G\alpha_{11}$, differentially recognized a p42 species, likely to be $G\alpha_{11}$, during lymphopoiesis (Fig. 1g), being detected in mature murine resting splenic B cells (lane 7), the human lymphoblastoid cell lines, Daudi (lane 3), Ramos (lane 2), Cess (lane 4) and Raji (weakly, lane 1), commonly used as model system for follicular B cells.[27,28,30] These results suggest that expression of $G\alpha_q$ and $G\alpha_{11}$, although generally considered to be ubiquitously expressed in mammalian tissues, may be differentially regulated in these

lymphopoietic cells with induction of $G\alpha_{11}$ correlating with increasing maturity of the B-cell lineage. This paucity of $G\alpha_{q/11}$ expression during lymphopoiesis is consistent with our earlier study reporting the absence of $G\alpha_{11}$ from human platelets and a few other haemopoietically-derived cell lines.[37]

Although $G\alpha_{11}$ expression is seen in the human leukaemic T-cell line, Jurkat (lane 9), there is no expression of $G\alpha_{q/11}$ in murine thymocytes or splenic T cells: these results obtained with primary T cells are consistent with the findings of Wilkie *et al.* [35] who reported that although $G\alpha_q$ mRNA was expressed widely in mammalian tissues, it was not detected in most T-cell lines tested. The relevance of $G\alpha_{11}$ expression in Jurkat T cells is therefore unclear: moreover, although Jurkat T cells are frequently used as a model of T-cell activation.[38,39] the precise phenotype of Jurkat T cells is still in some doubt as the original characterization of Jurkat T cells suggested that these cells may have been of thymic origin on account of the stability of their SRBC-rosette formation.[40] Interestingly, reverse transcription–polymerase chain reaction (RT–PCR) analysis suggests that human thymocytes express $G\alpha_s$, $G\alpha_{i1-3}$, $G\alpha_z$, $G\alpha_{q/11/16}$ and $G\alpha_{12/13}$, at least at the mRNA level.[41] However, we find that murine thymocytes, splenic T cells and human tonsillar T cells show little or no protein expression of any of the $G\alpha$ isoforms tested, except $G\alpha_s$, a finding consistent with the fact, that to date, there is no convincing data for G protein-regulated coupling of the T-cell receptor (TCR) or accessory receptors to lipid signalling pathways in primary T cells. In contrast, expression of $G\alpha_i$ and $G\alpha_{11}$ is quite high in Jurkat T cells and there is a recent report that $G\alpha_{11}$ is physically associated with, and functionally couples the TCR to PLC-β activation in these cells:[42] it is therefore possible that the generally high expression of $G\alpha$ subunits in Jurkat T cells, which rather mirrors the elevated $G\alpha_{i/11}$ expression in the human B lymphoblastoid cell lines, may simply reflect the transformation processes involved in immortalizing these B- and T-cell lines.

The paucity of $G\alpha_q$ and $G\alpha_{11}$ expression during early lymphoid differentiation may suggest that in these cells, effector functions generally thought to be regulated by $G\alpha_q$ and $G\alpha_{11}$ such as the β-isoforms of PLC, may be differentially regulated by $G\alpha_{15/16}$. Consistent with this, $G\alpha_{16}$ is strongly expressed in most of the human haemopoietic cells tested (Fig. 1i) with particularly high expression in the human pre-B-cell lines, REH, Nalm 6 and 697 (lane 6, results not shown and ref. 26). Interestingly, there is a progressive and marked down-regulation of expression correlating with differentiation through mature resting B cells to 'follicular' B cells (Daudi and Ramos, lanes 2 and 3). Except for the rather high expression of $G\alpha_{16}$ observed in the Raji cells, these results are broadly in line with recent RT–PCR analysis of $G\alpha_{16}$ expression in human neoplastic B-cell lines[26] which concluded that this G protein is likely to be involved in progenitor B-cell signalling as it expressed during early B-cell ontogeny and down-regulated during differentiation. It should be noted, however, that $G\alpha_{16}$ is not expressed in murine lymphoid cells of any maturation stage or lineage tested (lanes 8, 10 and 12), a finding perhaps consistent with the proposal that the closely related G protein, $G\alpha_{15}$ is the murine homologue of $G\alpha_{16}$.[35] These results showing differential expression of Gq-like α-subunits during lymphocyte development may suggest that, at discrete maturation stages of lymphoid cells, immunoreceptors may differentially couple to various PLC-β isoforms by distinct maturation-restricted Gq-family α-subunits. This proposal may be supported by recent data showing that whilst $G\alpha_{q/11}$ isoforms can activate PLC-$\beta 1 = \beta 3 \geqslant \beta 4 \geqslant \beta 2$, $G\alpha_{16}$ preferentially activates PLC-$\beta 2$.[43] Moreover, it has now emerged that whilst $\beta 1$ does not appear to be expressed in haemopoietic cells, expression of both PLC-$\beta 2$ and $G\alpha_{16}$ appears to be restricted to haemopoietic cells, again providing further support for atypical regulation of PLC-β isoforms by $G\alpha_q$ α-subunits at various stages of lymphoid development (reviewed in ref. 4). In addition, the generally low expression of $G\alpha_{q/11}$ isoforms may also reflect the fact that most of the immunoreceptors coupled to PLC activation in lymphoid cells appear to utilize $G\alpha_i$- and/or tyrosine kinase-regulated phospholipase C-γ isoforms.[44,45]

Finally, B and T cells were screened with an antiserum, BN3 which recognizes the $\beta_{1/2}$ G-protein subunits, previously believed to be virtually ubiquitously expressed at the mRNA level in mammalian tissues. The results obtained (Fig. 1j), however, showed that whilst $\beta_{1/2}$ subunits were generally strongly expressed in Jurkat T cells (lane 9), murine thymocytes (lane 11), some human B lymphoblastoid cell lines (lanes 1, 3 and 4), and in particular, in human pre-B-cell lines (lanes 5, 6), there was little or no expression in murine immature (lanes 8, 12) or mature splenic B cells (lane 7) or indeed, splenic T cells (lane 10). Although this finding could simply reflect maturation-restricted expression of G-protein β subunits, it was initially rather surprising as there is good evidence for pertussis toxin-sensitive (and hence $\beta\gamma$) coupling of the B-cell antigen receptors to PLC activation.[44,45] However, recent data have suggested that a $\beta 2\gamma 3$ complex was less effective at stimulating turkey erythrocyte PLC-β activity than other $\beta\gamma$ complexes,[46] suggesting that $\beta\gamma$-stimulation of PLC in B cells may indeed be maturation-restricted and involve expression and activation of one or more of the less well-characterized β-subunits (reviewed in ref. 4).

In conclusion, these studies have profiled G-protein expression during B- and T-cell development (Table 1) and confirmed that there is indeed differential expression of G-protein α- and β-subunits during lymphopoiesis. Although there is no clear pattern of maturation-associated differences with $G\beta_{1/2}$, $G\alpha_s$ and $G\alpha_{i2/3}$, there is a general correlation of decreased $G\alpha_s$ and increased $G\alpha_i$ isoform expression with activated or proliferating B and T cells: these findings may suggest that altered G-protein expression may provide a mechanism for suppressing the antiproliferative signal, cAMP, in activated or transformed lymphoid cells. In contrast to the rather maturation state-independent expression of $G\alpha_{s/i2/3}$, $G\alpha_{i1}$ was not expressed at the protein level in pre-B cells, immature and mature B cells or indeed, thymocytes or mature T cells. However, the finding that $G\alpha_{i1}$ appears to be induced only in the mature human B lymphoblastoid cell lines, which serve as models for follicular B cells,[27,28,30] may suggest that expression of this isoform correlates with the later stages of B-cell development. $G\alpha_{11}$ has a rather similar profile of expression to $G\alpha_{i1}$, being progressively induced during the transition of mature to germinal centre B cells. In contrast, $G\alpha_{16}$ shows an inverse pattern of expression in lymphoid cells, being most highly expressed in T cells and pre-B cells and expression then declining with B-cell maturation (Table 1; Fig. 1i and ref. 26). Thus, the contrasting profiles of $G\alpha_{i1/11}$

and $G\alpha_{16}$ serve not only as markers which can differentiate B- and T-cell maturation status but also suggest that PLC-signalling in lymphoid cells is differentially regulated in a maturation- and lineage-dependent manner.

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