T cell phenotypes in patients with common variable immunodeficiency disorders: associations with clinical phenotypes in comparison with other groups with recurrent infections

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Summary

Common variable immunodeficiency disorders (CVID) are a group of heterogeneous conditions that have in common primary failure of B cell function, although numerous T cell abnormalities have been described, including reduced proliferative response and reduced regulatory T cells. This study compared the T cell phenotype of CVID patients subdivided into clinical phenotypes as well as patients with partial antibody deficiencies [immunoglobulin (Ig)G subclass deficiency and selective IgA deficiency], X-linked agammaglobulinaemia (XLA) and healthy and disease controls. Absolute numbers of T cell subpopulations were measured by four-colour flow cytometry: naive T cells, central and effector memory and terminally differentiated (TEM) T cells, using CD45RA and CCR7 expression. Early, intermediate and late differentiation status of T cells was measured by CD27/CD28 expression. Putative follicular T cells, recent thymic emigrants and regulatory T cells were also assessed. Significant reduction in naive CD4 T cells, with reduced total CD4 and recent thymic emigrant numbers, was observed in CVID patients, most pronounced in those with autoimmune cytopenias or polyclonal lymphoproliferation. These findings suggest a lack of replenishment by new thymically derived cells. CD8 naive T cells were reduced in CVID patients, most significantly in the autoimmune cytopenia subgroup. There was a reduction in early differentiated CD4 and CD8 T cells and increased CD8 TEM in the CVID patients, particularly autoimmune cytopenia and polyclonal lymphoproliferation subgroups, suggesting a more activated T cell phenotype, due perhaps to an antigen-driven process. XLA patients had significantly reduced putative follicular T cells, which may depend on B cells for survival, while no significant alterations were observed in the T cells of those with IgG subclass deficiency or selective IgA deficiency.

Keywords: CVID, memory cells, primary antibody deficiency, T cells, XLA

Introduction

Common variable immunodeficiency disorders (CVID) are heterogeneous conditions that make up the most common group of clinically significant primary antibody deficiency (PAD). Patients with CVID are characterized by increased susceptibility to recurrent bacterial infection, coupled with low serum immunoglobulin levels and reduced specific antibody production in response to vaccination [1]. Patients may also have numerous clinical complications, including enteropathy, lymphoid malignancy, granuloma and autoimmunity, which have been used recently to classify patients into clinical phenotypes with varying prognoses [2,3]. CVID probably represent a polygenic group of primary antibody deficiency disorders of unknown aetiology [4].

Other PADs include X-linked agammaglobulinaemia (XLA), immunoglobulin (Ig)G subclass deficiency and selective IgA deficiency. Patients with XLA are profoundly antibody and B cell-deficient, and therefore experience recurrent bacterial infections [5]. However, they do not encounter the other clinical complications, common to many CVID patients, which are thought to relate to the underlying immune dysregulation. There have been suggestions that partial antibody deficiencies, in particular selective IgA

deficiency, may share a genetic basis with some types of CVID [6]. This is supported by reports of progression of selective IgA deficiency to CVID in rare patients [6].

Patients with CVID have a common feature in failure of B cell function, although a number of T cell abnormalities have been described, including reduced naive CD4 T cells [7], reduced proliferative responses to mitogens [8,9], reduced cytokine responses to mitogens and recall antigen [10,11] and reduced T regulatory cells (T_{regs}) [12–14] in selected patients. A subset of CVID patients are reported to have an increased susceptibility to recurrent viral infections or opportunistic infections that are more associated with T cell defects [7,15], particularly in those patients from consanguineous families [16], suggesting an unknown, autosomal recessive, combined immune deficiency.

Upon antigen encounter, naive T cells undergo a developmental pathway, resulting in the generation of central memory and effector memory T cells [17]. They can be measured in blood by use of the accepted markers, CCR7 and CD45RA [18]. In the early stages of differentiation, T cells express high levels of co-stimulatory molecules CD28 and CD27, which are lost sequentially upon differentiation [19,20]. CD31 and CD45RA co-expression is used to define recent thymic emigrants and correlates well with T cell receptor excision circle (T_{REC}) levels [21]. Follicular T cells are a distinct CD4 T cell lineage that regulates the development of antigen-specific B cell responses in the germinal centre and are identified by CXCR5 expression; putative follicular T cells in the periphery are identified by CD45RO and CXCR5 expression [22]. Percentages of these putative follicular T cells reduced in inducible T cell co-stimulator (ICOS) deficiency – a germinal-centre defect [23]. A recent study in CVID patients demonstrated that use of $CD127^{low}$ $CD25^+$ markers to discern T_{regs} correlated well with forkhead box protein 3 (FoxP3) expression [14]. These markers were utilized in this study.

T cell phenotypes have been investigated in a number of CVID cohorts, with reduction in CD4 naive T cells being the most consistent outcome [8,24,25]. However, the main limitation with most studies [24,26] was the heterogeneity of the CVID patient groups studied and the difficulties encountered in correlating laboratory phenotypes with clinically useful, defined clinical phenotypes. This study aimed to investigate a comprehensive range of T cell phenotypes in a large group of well-researched CVID patients in the context of their well-defined clinical phenotypes [2,3]. Also, for the first time, we have compared results from CVID patients with those from a disease control as well as a healthy control group. As a comparison, we also investigated the T cell phenotypes in other partial antibody deficiency groups and XLA. To our knowledge, this paper investigates the most comprehensive selection of T cell subsets of all papers published so far, including CD45RA, CCR7 to distinguish naive, effectors, central memory and terminally differentiated T cells; CD28/CD27 co-stimulation markers to determine dif-

ferentiation state (not published in antibody deficiency groups to our knowledge); and recent thymic emigrants, putative follicular T cells and T_{regs}.

Materials and methods

Study population

Controls and patient groups were recruited to this study through the Clinical Immunology Department at the John Radcliffe Hospital, Oxford, UK under the ethical approval of the Central Oxfordshire Research Ethics Committee (05/Q1605/88). All subjects gave informed, written consent and the studies were performed according to the Declaration of Helsinki.

All patients used met international diagnostic criteria [Pan-American Group for Immunodeficiency (PAGID) and European Society for Immunodeficiencies (ESID)], and included 58 CVID patients, 15 IgG subclass with IgA-deficient patients (Gsub), 14 IgA-deficient patients (IgA) and nine XLA patients. Healthy controls were recruited from hospital staff to match the age range and gender bias of the total CVID group (see Table 1 for study group demographics). Healthy controls were individuals aged 18 years or over willing to donate blood who passed our exclusion criteria. Exclusion criteria included pregnant women, any medical condition which affects the immune system (for example, autoimmunity, recurrent infections, immunodeficiency, lymphoproliferative disease) or treatment with a drug known to affect the immune system.

In order to control for the effect of infection on the T cell subpopulations, disease controls were recruited from the immunodeficiency clinic. These were immune-competent patients who had an increased infection burden, in whom no clinical or laboratory evidence for immunodeficiency was found. Results from this group were included only once a period of 1 year had elapsed since discharge from the clinic, to rule out an evolving immunodeficiency. The immune tests undertaken were guided by clinical and family histories. The typical panel of tests performed included: IgG, IgA and IgM, and serum and urine electrophoresis with immunofixation if indicated. Specific antibody responses to the vaccines tetanus, pneumococcal and *Haemophilius influenza* B were performed, and if absent/low responses were noted the patient was vaccinated and these retested after 1 month. Lymphocyte subsets, both percentage and absolute count, were also performed, including measurement of B cells, CD4 and CD8 T cells and natural killer (NK) cells [3,27].

At the time of analysis, all XLA and 55 of 58 CVID patients were on immunoglobulin replacement, but not on immuno-suppressive therapy. Those with autoimmune cytopenia or lymphoid interstitial pneumonia had not received corticosteroid therapy within 6 months, and only at prior doses <25 mg/kg. No patient had an affected parent, sibling or child.

Table 1. Demographics of the study groups.

	Number	Male (% male)	Age (years), median and range	*P*-value	Age of presentation (years), median and range
Healthy controls	48	17 (35·4%)	43·4 (21·4–84·6)	n.s.	–
Disease controls	31	10 (32·3%)	42·7 (18·5–73·8)	n.s.	–
IgG subclass deficiency	15	8 (53·3%)	36·4 (15·6–66·8)	n.s.	14·5 (2–58)
Selective IgA deficiency	14	5 (35·7%)	32·9 (13·5–63·7)	n.s.	37 (13–63)
XLA	9	9 (100%)	32·0 (0·5–56·17)	$P < 0.05$	1 (0·5–5)
CVID total	58	28 (48·3%)	46·4 (17·3–75·2)	n.s.	25 (0·75–63)
Infection only	29	14 (48·3%	39·9 (17·3–75·2)	n.s.	18 (1–55)
Polyclonal lymphoproliferation	9	5 (55·6%)	54·2 (34·5–67·3)	n.s.	31 (13–54)
Cytopenias	10	4 (40%)	50·0 (20·0–75·1)	n.s.	28 (0·75–62)
Organ-specific autoimmunity	20	11 (55%)	51·4 (20·0–75·1)	n.s.	31 (0·75–63)
Lymphoid malignancy	1	1 (100%)	55·4	–	28
Enteropathy	3	2 (66·7%)	54·5 (44·4–55·4)	–	27 (20–28)

Age distribution of groups was compared using one-way analysis of variance with Tukey's multiple comparison test as a *post-hoc* test. CVID: common variable immunodeficiency disorder; Ig: immunoglobulin; n.s.: not significant; XLA: X-linked agammaglobulinaemia.

CVID patients were categorized into the following clinical phenotypes, as described in Chapel *et al.* [2,3]: infection only (IO), enteropathy, lymphoid malignancy, polyclonal lymphoproliferation (PL), organ-specific autoimmune disease (OSAI) or autoimmune cytopenias (AC) which included immune thrombocytopenia (ITP). ITP is defined as platelets $<100 \times 10^9/l$, persistent (>6 months), one episode treated with steroids [3]. The autoimmune diseases in patients in the OSAI group included: autoimmune thyroid disease ($n = 5$), psoriasis ($n = 6$), uveitis ($n = 2$), vitiligo ($n = 2$), pernicious anaemia ($n = 3$), ulcerative colitis ($n = 4$) and type 1 diabetes ($n = 2$). Only one patient had a subsequent lymphoid malignancy and only three had an enteropathy, so these categories were not utilized in the analysis; these patients were included in the CVID total group. Figure 1 demonstrates the distribution of clinical phenotypes of the CVID patient group.

The number of patients stated in each group in Table 1 is the maximum number of patients analysed for a T cell subpopulation. However, for some of the T cell subpopula-
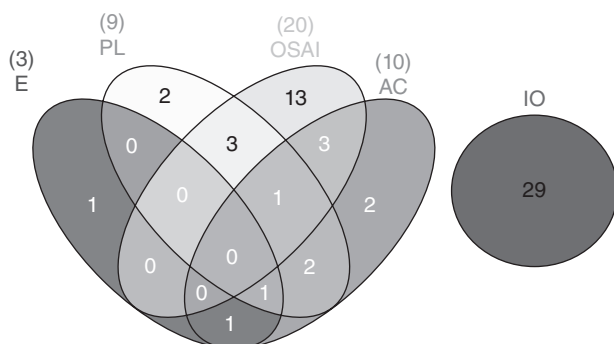

Fig. 1. Venn diagram illustrating the distribution of common variable immunodeficiency disorder (CVID) patients into clinical phenotypes [2,3]. Numbers in brackets indicate numbers in each division; patients may appear in more than one group, as indicated. E: enteropathy; PL: polyclonal lymphoproliferation; AC: autoimmune cytopenias; OSAI: organ-specific autoimmune disease; IO: infection only.

tions smaller numbers were analysed due to either technical difficulties with a particular tube or limited sample availability.

Flow cytometry

All flow cytometric analysis was performed on ethylenediamine tetraacetic acid (EDTA) blood samples within 48 h of venepuncture. Lymphocyte subset analysis and absolute counts of total lymphocytes, total T cells, CD4 and CD8 T, B and NK cells were performed using BD Multitest™ CD3/CD16⁺CD56/CD45/CD19 and CD3/CD8/CD45/CD4 with BD Trucount™ Tubes (Becton-Dickinson, San Jose, CA, USA) and acquired on a BD fluorescence activated cell sorter (FACS)Calibur (Becton Dickinson), as per the manufacturer's instructions.

For T cell subpopulations, 100 μl of whole blood was incubated with directly conjugated fluorescent antibodies for 30 min in the dark at room temperature, then red cells were lysed using FACSlyse (Becton-Dickinson), washed in phosphate-buffered saline (PBS) and fixed in PBS with 1% formaldehyde. Samples were acquired using four-colour acquisition on a FACSCalibur and data analysed using CellquestPro software (Becton-Dickinson). Fluorescence minus one gating techniques were employed to evaluated thresholds for positivity of individual antibodies and aid gating of T cell subpopulations. The following CD3⁺ T cell subpopulations were analysed on CD4 and CD8 cells: naive, central memory (CM), effector memory (EM) and terminally differentiated determined (TEM) by CCR7 and CD45RA expression; early, intermediate and late differentiation status was determined by CD28/CD27 expression. Other CD4 T cell populations included recent thymic emigrant (defined by CD45RA/CD31), putative follicular T cells (defined by CXCR5/CD45RO) and T$_{regs}$ (defined by CD25⁺CD127⁻). Absolute cell counts were calculated using the CD4 or CD8 T cell counts from the lymphocyte subset

analysis. Subpopulations were added together to ensure that the total number of CD4 or CD8 matched those from the lymphocyte subset analysis.

Statistical analyses

All statistical analyses were performed using GraphPad Prism version 4 (GraphPad Software, San Diego, CA, USA). All data were analysed using non-parametric one-way analysis of variance (ANOVA) Kruskal–Wallis with Dunn's multiple comparison test as a *post-hoc* test or one-way ANOVA with Tukey's multiple comparison test as a *post-hoc* test. T cell subpopulation correlations with age were analysed by Spearman's correlation. The Venn diagram (Fig. 1) was made using the J.C. Oliveros (2007) VENNY tool from http://bioinfogp.cnb.csic.es/tools/venny/index.html

Results

Comparison of T cell populations with age

It was important to age- and gender-match the healthy controls and patient groups, as an effect of age on some T cell subpopulations has been described [21]. This was performed successfully, except for the XLA patients, who were significantly younger and all male (Table 1).

There were no significant differences observed between the disease controls (*n* = 31) and healthy controls (*n* = 48) in any of the T cell subpopulations studied (see Figs 3 and 4), so the two control groups were combined to determine whether or not there were any relationships between T cell subpopulations and age. No significant correlations between age and any T cell subpopulation were observed in this age range (18·5–84·6years at the time of study) (all *r²* values were less than 0·5 or −0·5).

Patient demographics and clinical phenotypes of the CVID group

Table 1 describes the demographics of the subjects studied and controls, including age of presentation. Partial antibody deficiency groups IgG subclass (*n* = 15) and selective IgA deficiency (*n* = 14), as well as XLA (*n* = 9) were included for comparison with CVID patients (*n* = 58). CVID patients were not included if they had suffered opportunistic infections. Figure 1 demonstrates the clinical phenotypes of the CVID patient group. Of the 58 CVID patients studied, 50% had infections only, with no other disease-related complications, while 34% had OSAI, 17% had AC, 16% had PL and 5% had enteropathy. Sixty-two per cent of CVID patients with complications had only one complication; Figure 1 indicates the overlap of complications within the patient group. Patients with more than one complication appear in all relevant subgroups in the figures.

Comparison of T cell subpopulation absolute counts in PAD

Lymphocyte subset analysis demonstrated that patients with CVID overall have significantly lower total CD4 T cells numbers compared with both control groups (*P* < 0·001; Fig. 2), while there was no significant difference in CD8 T cell numbers (data not shown).

CD4 T cell subpopulation absolute counts

Table 2 summarizes the T cell subpopulation absolute counts in the PAD groups and controls. Figure 3a shows significantly lower CD4 naive T cell absolute numbers in the CVID total group compared to the disease and healthy controls groups (*P* < 0·001). When the CVID patients were subdivided into clinical phenotypes, the AC and OSAI groups had the most significantly reduced number of CD4 naive T cells (*P* < 0·001), followed by the PL group (*P* < 0·01), when compared to both control groups (see Fig. 3a). Within CD4 memory subpopulations CD4 CM and the CD4 EM cells demonstrated a significant difference between groups (Fig. 3b,c). The CD4 CM cells were reduced in the AC group compared to both control groups (Fig. 3b, *P* < 0·01).

The CVID total group, and most markedly the OSAI group, demonstrated significantly lower numbers of CD4 T
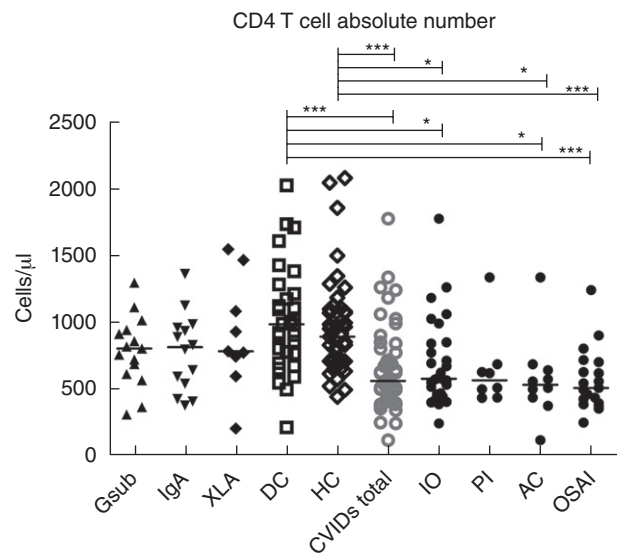

Fig. 2. CD4 T cell counts (cells/μl). Common variable immunodeficiency disorder (CVID) patients may appear in more than one group, as indicated in Fig. 1. Gsub: immunoglobulin (Ig)G subclass-deficient; IgA: IgA-deficient; XLA: X-linked agammaglobulinaemia; DC: disease control; HC: healthy control; CVID all subjects with CVID; IO: infection only; PL: polyclonal lymphoproliferation; AC: autoimmune cytopenias; OSAI: organ-specific autoimmune disease. Statistics were performed with one-way analysis of variance (ANOVA) Kruskal–Wallis, with Dunn's multiple comparison test as a *post-hoc* test. *P* < 0·05; ***P* < 0·001.

Table 2. T cell subpopulations expressed as absolute cell counts (cells/μl).

	IgG subclass Cells/μl	IgA Cells/μl	XLA Cells/μl	DC Cells/μl	HC Cells/μl	CVID total Cells/μl	IO Cells/μl	OSAI Cells/μl	AC Cells/μl	PL Cells/μl	ANOVA result
CD4 CD45RA+ CCR7+ naive	58–748	130–827	103–1046	1–1262	54–1322	3–1162	24–667	20–408	3–1162	16–1162	<0·0001
	287	340	553	385	325	113	189	84	38	44	
CD4 CD45RA− CCR7+ central memory	127–430	96–520	68–411	0–832	140–920	58–1049	64–1049	58–640	58–289	58–454	0·003
	275	263	201	302	312	254	256	261	161	234	
CD4 CD45RA− CCR7− effector memory	32–352	51–215	28–183	0–727	42–436	6–385	64–385	25–342	6–364	6–364	0·046
	189	104	81	195	154	133	114	170	253	184	
CD4 CD45RA+ CCR7− terminally differentiated	1–73	4–37	0–20	0–63	1–134	0–101	1–101	0–26	0–100	1–100	n.s.
	10	10	7	12	9	7	7	5	7	9	
CD8 CD45RA+ CCR7+ naive	31–395	18–274	18–230	16–460	15–447	14–500	21–586	20–225	14–117	21–91	0·0001
	147	169	119	100	125	78	86	69	68	66	
CD8 CD45RA− CCR7+ central memory	6–62	9–112	15–47	3–149	12–128	2–306	8–174	2–306	2–306	2–57	0·011
	24	23	20	46	37	44	44	53	23	22	
CD8 CD45RA− CCR7− effector memory	40–520	67–428	95–376	28–736	69–714	9–700	28–654	18–700	9–239	9–239	0·026
	199	146	207	152	226	131	161	114	143	131	
CD8 CD45RA+ CCR7− terminally differentiated	11–339	13–530	14–181	16–257	12–986	6–461	6–461	12–314	21–344	48–344	0·0293
	74	79	38	40	77	89	73	74	166	181	
CD8 CD28+ CD27+ early differentiation	75–599	88–458	98–318	32–857	107–772	32–1081	95–1081	46–380	32–380	46–263	<0·0001
	301	324	249	308	335	194	226	196	181	160	
CD8 CD28− CD27+ intermediate differentiation	15–114	12–215	15–146	2–272	11–271	7–178	9–169	7–178	19–121	21–121	n.s.
	37	42	58	33	44	45	51	31	57	60	
CD8 CD28− CD27− late differentiation	5–402	8–349	13–321	2–284	7–557	2–703	5–556	2–703	29–329	20–329	n.s.
	63	46	40	40	77	90	80	77	130	153	
CD4 CD28+ CD27+ early differentiation	287–1220	338–1291	184–1493	1–1916	376–1980	87–1673	198–1673	160–1111	87–1156	263–1156	<0·0001
	706	750	749	894	756	453	487	438	410	488	
CD4 CD28+ CD27− intermediate differentiation	14–90	19–157	16–78	0–211	25–174	11–201	16–151	11–123	11–201	19–148	n.s.
	40	30	37	46	53	47	50	34	45	89	
CD4 CD28− CD27− late differentiation	0–294	0–74	0–16	0–133	0–208	0–228	0–228	0–209	1–51	0–51	n.s.
	2	4	0	1	2	3	2	0	6	11	
T$_{regs}$ CD4+ CD25^{hi} CD127−	15–88	15–81	13–110	0–136	22–104	3–150	5–104	3–150	8–42	6–41	<0·0001
	24	56	41	43	50	36	44	30	27	17	
Thymic emigrant CD45RA CD31+	65–645	73–537	74–494	0–651	24–824	1–409	9–409	8–287	1–163	13–163	<0·0001
	173	248	411	211	208	87	116	68	47	53	
Putative follicular T cells CXCR5+ CD45RO+	47–219	72–172	0–52	0–204	54–288	5–417	38–346	5–247	12–417	92–417	<0·0001
	105	110	32	87	95	111	108	136	136	133	

Median and range are indicated for all groups, as well as result of Kruskal–Wallis one-way analysis of variance (ANOVA). AC: autoimmune cytopenias; CVID: common variable immunodeficiency disorder; DC: disease control; HC: healthy control; Ig: immunoglobulin; IO: infection only; OSAI: organ-specific autoimmune disease; PL: polyclonal lymphoproliferation; T$_{reg}$: regulatory T cells; XLA: X-linked agammaglobulinaemia.

cells at an early differentiation stage expressing both the co-stimulatory molecules CD28/27, compared to both control groups ($P < 0.001$) (Fig. 3d). The IO ($P < 0.05$) and AC groups ($P < 0.01$) also demonstrated significantly lower numbers of CD4 T cells expressing both the co-stimulatory molecules CD28/27 compared to both control groups. There was no compensatory increase in the numbers of CD4 T cells losing expression of either CD27 only or CD27/28 in the CVID subgroups (Table 2).

CD8 T cell subpopulation absolute counts

Significantly lower numbers of CD8 naive T cells were observed in the CVID total and AC groups compared to the healthy controls ($P < 0.01$ $P < 0.05$, respectively, Fig. 3e). Within the CD8 memory subpopulations, CD8 EM were significantly lower in number in OSAI compared to healthy controls ($P < 0.05$, Fig. 3f) and CD8 TEM were significantly higher in the PL and AC groups compared to disease controls ($P < 0.05$, Fig. 3g). This was accompanied by a significantly lower number of CD8s at an early differentiation stage co-expressing CD28 and CD27 compared to the healthy control group in the overall CVID group ($P < 0.001$), the PL and OSAI subgroups ($P < 0.01$) and the AC subgroup ($P < 0.05$) (Fig. 3h). There was no compensatory increase in CD8 T cells losing expression of either CD27 only or CD27/28 in the CVID subgroups (Table 2).

Recent thymic emigrants, T_{regs} and putative follicular T cells

Absolute numbers of recent thymic emigrants were decreased significantly in the CVID total group ($P < 0.001$) compared to the healthy control group, and were particularly decreased in the OSAI ($P < 0.01$), PL and AC subgroups ($P < 0.05$, Fig. 4a). The number of T_{regs} was significantly lower in CVID total group ($P < 0.01$) and in the OSAI, AC and PL subgroups ($P < 0.001$, $P < 0.05$ and $P < 0.05$, respectively) compared to healthy controls (Fig. 4b). The numbers of putative follicular T cells were altered significantly only in the XLA group (Fig. 4c), which were significantly lower than the healthy control group ($P < 0.05$).

T cell subpopulation absolute counts in partial antibody deficiencies and XLA patients

There were no significant differences in absolute cell counts between either the IgG subclass deficiency or IgA deficiency groups and either control groups in any of the CD4 or CD8 T cell subpopulations (Figs 3 and 4). However, there were significant differences in the XLA group compared to the healthy control group, including significantly lower numbers of CD4 effector T cells ($P < 0.05$, Fig. 3c), accompanied by a trend for higher numbers (Fig. 3a) of CD4 naive T cells and recent thymic emigrants (Fig. 4a). There was a significant
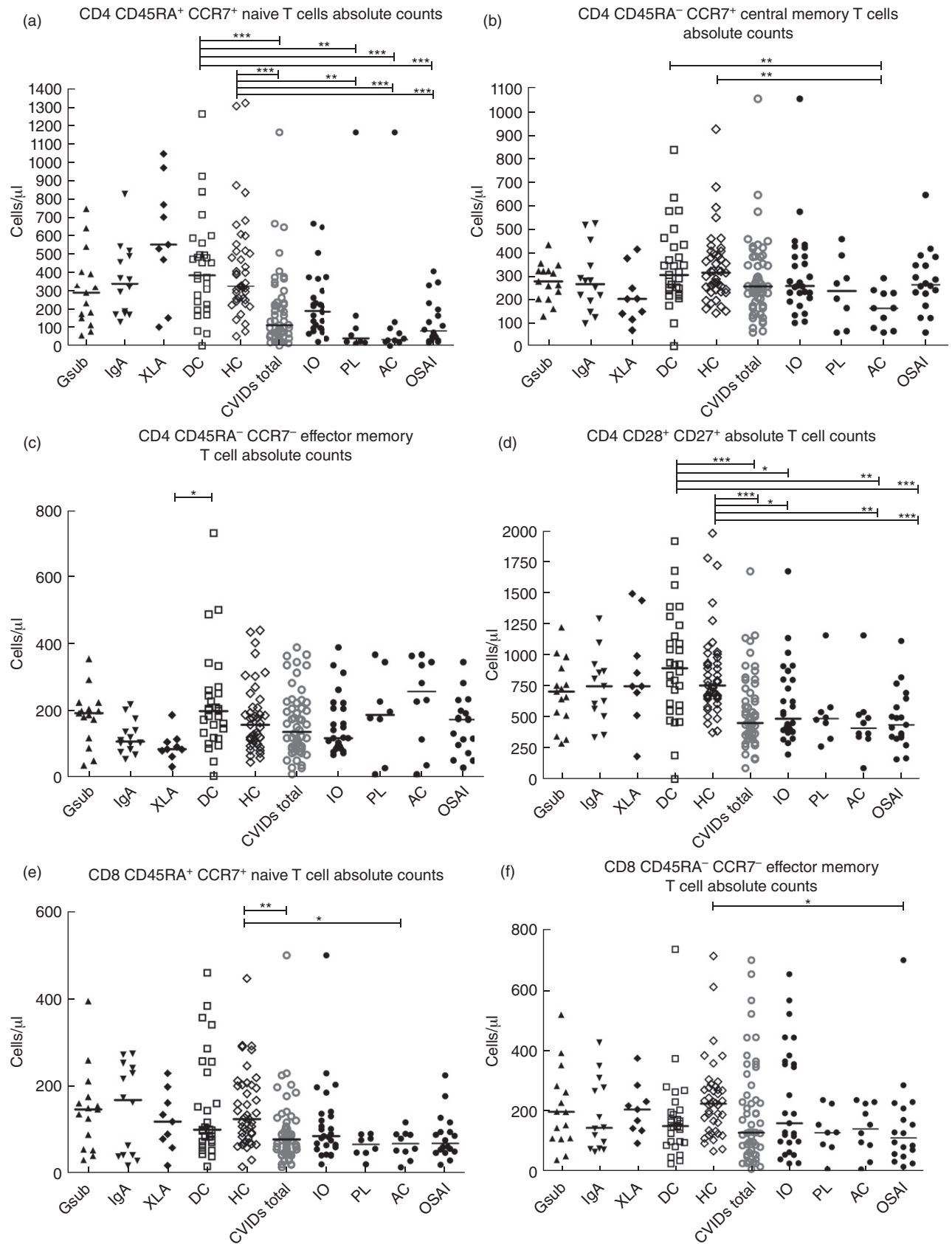
decrease in numbers of putative follicular T cells in the XLA group compared to healthy controls ($P < 0.05$, Fig. 4c).

Discussion

This was a large one-centre study comparing absolute numbers of a comprehensive range of T cell subpopulation phenotypes in a well-defined group of patients with validated diagnoses of CVID and well-documented complications. The results were compared with those from 38 patients with XLA or partial antibody deficiencies, and with age-matched healthy or disease controls. We have found that a number of T cell subpopulations are altered in patients with CVID or XLA, compared to partial antibody deficiencies and both control groups.

The total CD4 numbers in CVID patients were reduced significantly compared to controls, as in other reported cohorts. This probably accounts for the reduction in CD4/8 ratio and increased CD8 percentages observed in a proportion of CVID patients [7,12,24], particularly in the subgroup with opportunistic infections [16]. The primary purpose of this study was to identify the changes in the absolute numbers of T cell subpopulations associated with different clinical CVID phenotypes. Naive CD4 T cell numbers were reduced significantly in CVID, specifically in the PL, AC and OSAI subgroups. This supports other reports [7,24], particularly from Mouillot et al. [25], who reported that CVID patients with lymphoproliferation or autoimmunity demonstrated the most profound reduction in CD4 naive T cells. Thymic output of new T cells is known to correlate negatively with age [21], and therefore age-matching of the control groups was important to minimize the impact. In addition, thymic cells are particularly sensitive to corticosteroids, so it was important to use a disease control group of immune-competent patients referred to the clinic with recurrent infections.

Recent thymic emigrant numbers were also reduced significantly in CVID patients, specifically in the PL, AC and OSAI subgroups; CVID patients with such complications treated with corticosteroids were excluded if they had received such therapy within 6 months of analysis. Together with the reduced CD4 naive T cells, reduced thymic emigrants suggest a lack of replenishment of the CD4 T cell pool by new thymically derived cells in CVID patients. Giovannetti et al. [24] also found that thymic output was reduced significantly in CVID patients, and associated this with a reduction in class-switch memory B cells, expansion of CD21^{lo} B cells, splenomegaly and granuloma. They also showed increased cell turnover as measured by Ki-67, particularly in the CD4 naive subset and increased apoptosis [24]. We did not find such an association with CD21^{low} B cells, although we found an association with PL for which granuloma is a criterion. Mouilott et al. [25] found a decrease in CD4 naive T cells which was accompanied by increased CD95$^+$ expression, most pronounced in the PL
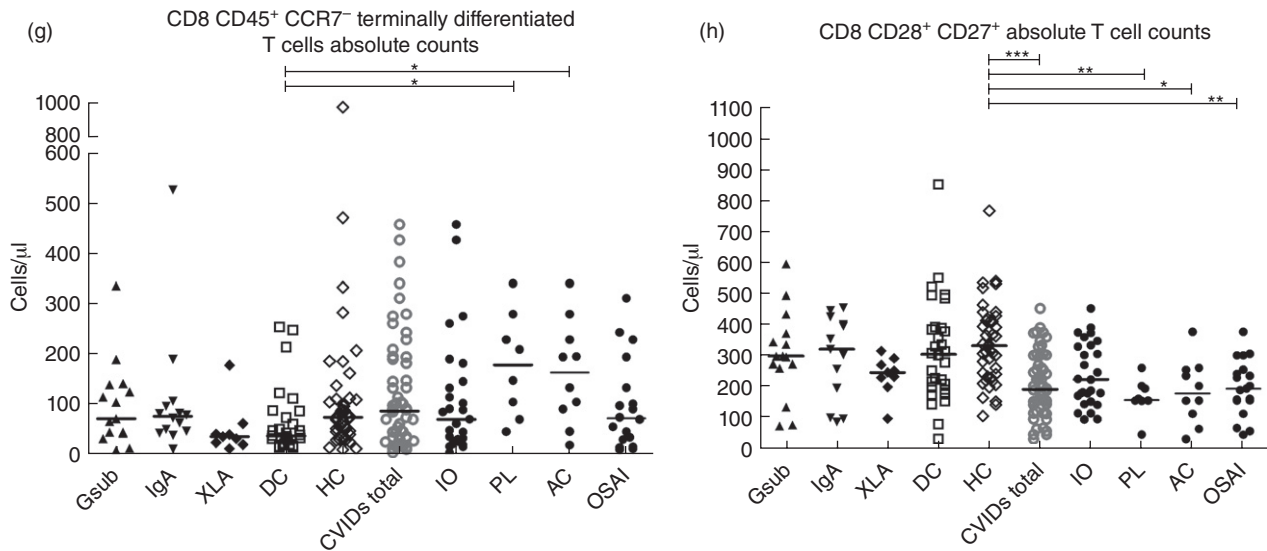
(a) CD4 CD45RA+ CCR7+ naive T cells absolute counts

(b) CD4 CD45RA− CCR7+ central memory T cells absolute counts

(c) CD4 CD45RA− CCR7− effector memory T cell absolute counts

(d) CD4 CD28+ CD27+ absolute T cell counts

(e) CD8 CD45RA+ CCR7+ naive T cell absolute counts

(f) CD8 CD45RA− CCR7− effector memory T cell absolute counts

Fig. 3. Comparison of T cell naive and memory subset absolute counts (cells/μl) (a) CD4 naive T cells, (b) CD4 central memory, (c) CD4 effector memory, (d) CD4 early differentiation, (e) CD8 naive T cells, (f) CD8 effector memory, (g) CD8 terminally differentiated, (h) CD8 early differentiation. Common variable immunodeficiency disorder (CVID) patients may appear in more than one group, as indicated in Fig. 1. Gsub: immunoglobulin (Ig)G subclass-deficient; IgA: IgA-deficient; XLA: X-linked agammaglobulinaemia; DC: disease control; HC: healthy control; CVID all subjects with CVID; IO: infection only; PL: polyclonal lymphoproliferation; AC: autoimmune cytopenias; OSAI: organ-specific autoimmune disease. Statistics performed with one-way analysis of variance (ANOVA) Kruskal–Wallis, with Dunn's multiple comparison test as a *post-hoc* test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

and AC groups, while Iglesias *et al.* [28] found that CD4+CD45RA+ T cells, which contain predominantly naive CD4 T cells, had increased spontaneous apoptosis and CD95 expression in CVID patients. Therefore, the reduction in naive CD4 T cells may, in part, be due to both reduced thymic output and increased cell turnover.

Significant reductions in CD8 naive T cell numbers were seen in CVID patients compared to controls, particularly in the AC group. This has not been reported previously, and is likely to reflect the increases in terminally differentiated CD8 cells observed in the PL and AC groups.

Both CD4 and CD8 T cells in CVID patients, and most significantly in the AC, OSAI and PL groups, demonstrated a loss of the co-stimulatory molecules CD28 or CD27. This suggests T cell differentiation along an activation pathway. Other groups have observed increased activation in T cells of all CVID patients [25], as measured by CD38 and human leucocyte antigen D-related (HLA-DR) [24], particularly in patients with splenomegaly [26]. The possibility of an infectious agent driving the clinical manifestations of lymphoproliferation observed in the PL subset of CVID patients has been suggested, but not established – a hypothesis supported by these T cell phenotypes. It has been suggested that cytomegalovirus (CMV) may play a role in the T cell abnormalities seen in CVID, as patients in one study had a 13-fold increased proportion of CMV-specific, functional T cells compared to aged-matched controls [29]. CMV-specific CD8 T cells have the phenotype of CD45RA+CCR7−CD27−

and the increase in CD8 T cells of this phenotype in the PL and AC subgroups of the CVID suggests that CMV or another similar infectious agent may be important [17,30].

We found significantly increased percentages of putative follicular T cells in CVID patients (data not shown) although, interestingly, not increased absolute counts. This may reflect the lack of naive T cells altering the proportion of CD4 T cells, and suggests that the most accurate method of assessing lymphocyte phenotypes is by cell number, not percentage. There was a significant reduction in number of putative follicular T cells in XLA. Bossaller *et al.* [23] found reduced percentages of these putative follicular T cells in ICOS deficiency and suggested that such cells could be a marker for a functional GC in humans. Martini *et al.* [5] found CD4+CD45RO+ memory T cells and CD4+CD45RO+CXCR5+ putative follicular T cells to be reduced significantly in XLA patients, regardless of age. They also found these putative follicular T cells to be reduced significantly in CVID patients with <2% B cells, supporting the theory that the presence of B cells but not Btk is required for generation of these putative follicular T cells [5]. There was a larger range of putative follicular T cell number in patients with CVID compared to controls, suggesting that patients outside the normal range for these putative follicular T cells may warrant investigation for defects resulting in poor germinal-centre formation.

T$_{regs}$ were reduced significantly in number in CVID patients, most profoundly in PL, AC and OSAI patients, confirming previous work [13,14,25,31]. Arumugakani *et al.*
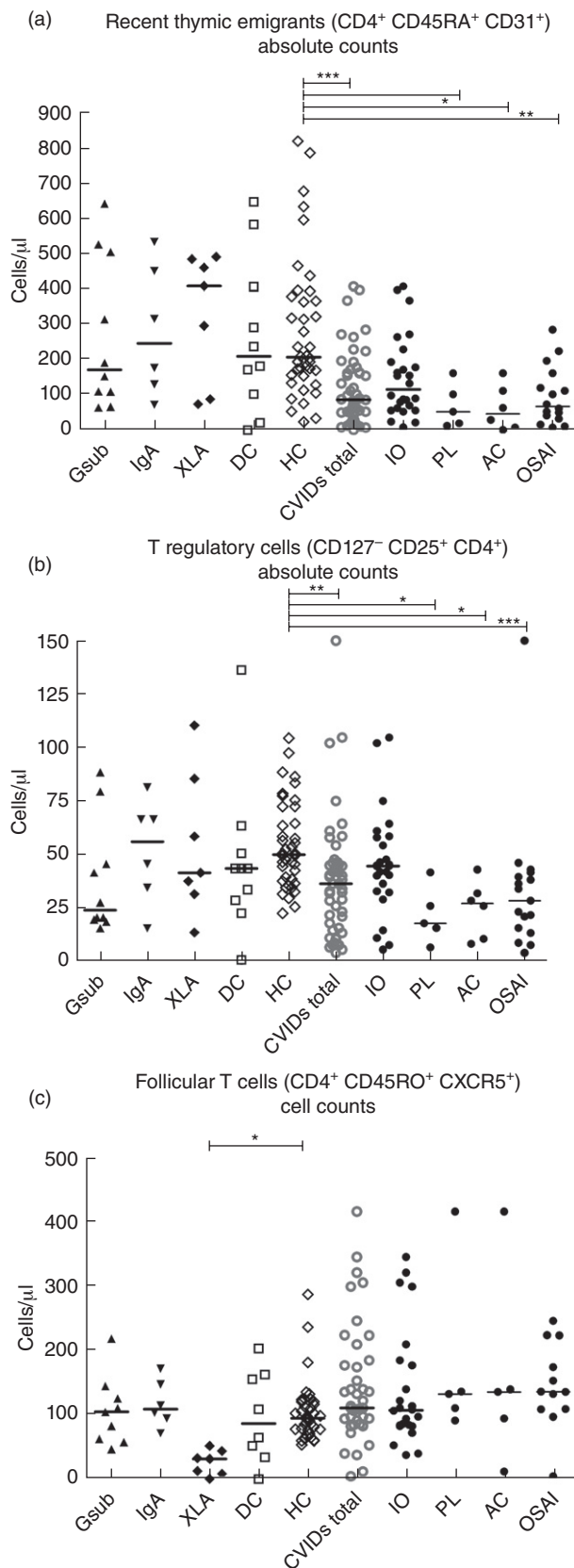
(a)

Recent thymic emigrants (CD4+ CD45RA+ CD31+) absolute counts

(b)

T regulatory cells (CD127− CD25+ CD4+) absolute counts

(c)

Follicular T cells (CD4+ CD45RO+ CXCR5+) cell counts

Fig. 4. Comparison of putative follicular T cell, recent thymic emigrants and regulatory T cells (T_{regs}) as absolute values (cells/μl) (a) CD4 T cell recent thymic emigrants, (b) T_{regs}, (c) CD4 putative follicular T cells. Common variable immunodeficiency disorder (CVID) patients may appear in more than one group as indicated in Fig. 1. Gsub: immunoglobulin (Ig)G subclass-deficient; IgA: IgA-deficient; XLA: X-linked agammaglobulinaemia; DC: disease control; HC: healthy control; CVID all subjects with CVID; IO: infection only; PL: polyclonal lymphoproliferation; AC: autoimmune cytopenias; OSAI: organ-specific autoimmune disease. Statistics performed with one-way analysis of variance (ANOVA) Kruskal–Wallis, with Dunn's multiple comparison test as a *post-hoc* test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

[12] found reduced $FoxP3^+$ T_{reg} numbers and percentages in CVID patients with autoimmunity and splenomegaly, and it was associated with an expansion of $CD21^{lo}$ B cells.

We found no significant differences in any T cell subpopulations in the partial antibody deficiency groups, namely IgG subclass or selective IgA-deficient. This supports the findings of Litzman *et al.* [32], who found no significant differences in a small range of T cell memory markers in selective IgA-deficiency patients compared to healthy controls. Our findings suggest no gross defect in T cell differentiation in these partial antibody deficiency groups. CVID patients with infections only demonstrated no significant differences in T cell subpopulations, except reduction in absolute numbers of CD4 T cells in the early differentiation stage (expressing CD28/27), suggesting that abnormalities in T cell subpopulations correlate with other complications such as autoimmunity, especially cytopenias and polyclonal lymphoproliferation, rather than being crucial for the pathogenesis of primary antibody failure.

In conclusion, there was a significant reduction in numbers of naive CD4 T cells in CVID patients, accompanied by a significant reduction in numbers of recent thymic emigrants, suggesting lack of replenishment of the CD4 T cell pool by new thymic-derived cells. CD8 naive T cells were also reduced, specifically in the AC subgroup, and were accompanied by an increase in terminally differentiated CD8s. There was a reduction in both early differentiated CD4 and CD8 T cells in the AC, OSAI and PL subgroups, demonstrating a more activated T cell phenotype, suggestive of an antigen-driven process.

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Disclosure

Authors' conflicts of interest: None declared.

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