

Antigen-Specific versus Total Immunoglobulin Synthesis: Total IgE and IgG1, but Not IgG2a Levels Predict Murine Antigen-Specific Responses

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Key Words

Mouse · Antibodies · Allergy · IgE, Th1/Th2

Abstract

Background: Induction of an effective antibody (Ab) response requires delivery of multiple signals to B cells. Cross-linking of the B cell antigen receptor (BCR), signaling through CD40 and CD80/86 and cytokine signals combine to induce class switching and expression of specific isotypes. These signals are principally derived from activated, antigen (Ag)-specific T cells. In contrast, IFN γ , the only cytokine known to induce class switch to IgG2a, can be produced systemically by activated NK or NKT cells, suggesting that Ag-nonspecific signals may also regulate IgG2a production. **Methods:** Given the potential differences in regulation between IgE/IgG1 versus IgG2a, we immunized mice on day 0 with ovalbumin (OVA) in the presence of strong type-1- or type-2-immunity-inducing adjuvants and boosted mice 4 weeks later. Mice were bled during the primary immune response and after boost to assess primary and recall Ab responses. **Results:** Regardless of strain of mice used, phenotype (type 1 versus type 2 dominated) or nature of the immune response induced (primary versus recall), strong correlations between OVA-specific and total IgE

and IgG1 were demonstrated. In contrast, a consistent lack of correlation between OVA-specific and total IgG2a levels was observed in all but BALB/c mice. **Conclusion:** These data indicate that the increase in total levels of IgE/IgG1 isotypes is primarily a result of increased levels of OVA-specific Ab. In contrast, the lack of correlation between total and OVA-specific IgG2a suggests broader activation of IgG2a-producing B cells routinely occurs following exogenous Ag immunization.

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Introduction

Generation of an effective antibody (Ab) response requires numerous signals including B cell antigen receptor (BCR) cross-linking, CD40 ligation and cytokine signals to initiate and direct Ig class switching. In mice, B cells may undergo class switch to either IgE or IgG1 following exposure to IL-4 or IL-13 [1–4]. Considerable evidence indicates that IgE production is more dependent upon IL-4 than is IgG1 production. IL-4-deficient mice have low to undetectable levels of IgE while levels of IgG1 remain substantial [5, 6] and IL-4 can induce germline ϵ , but not $\gamma 1$ transcripts in the absence of other signals [7–9]. Furthermore, T cell help in the form of CD40L-CD40 and CD28-CD80/86 interaction is required for optimal production of these isotypes. Individual B cells produce higher quantities of IgE or IgG1, but not other isotypes, fol-

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lowing stimulation through CD86 [10, 11]. While recent evidence indicates sCD14 may play an important role in lowering serum IgE levels [12, 13], the principal signals promoting class switch to, and optimal expression of IgE/IgG1 are delivered chiefly by activated T cells.

The only cytokine known to cause class switch to IgG2a is IFN γ [14]. By virtue of their ability to produce large quantities of IFN γ following activation, NK cells have been suggested to play an important role in IgG2a regulation. Although we and others have shown that acute depletion of NK 1.1+ cells has no demonstrable effect on antigen (Ag)-specific IgG2a production following immunization with T-independent or T-dependent Ags [15, 16], chronic depletion of NK cells resulted in almost complete abrogation of Ag-specific IgG2a responses [17]. Furthermore, it has been demonstrated that stimulation of NK-cell-derived IFN γ production through the use of poly I:C [15], or tumor cell lines [18] significantly increased Ag-specific IgG2a levels. Thus unactivated NK cells appear not to be involved in regulation of Ag-specific Ab responses, yet systemic activation of these cells of innate immunity by Ag-nonspecific signals significantly increases IgG2a responses.

The relationship between Ag-specific and total Ig levels in animal models has not been explicitly explored to date. Given the possibility that absolute levels of IgG2a and IgE/IgG1, reflecting prototypic type 1 versus type 2 effector responses, are regulated through different mechanisms, we determined the extent of correlation between Ag-specific and total Ig levels of these isotypes in exogenous Ag-driven responses. If, following immunization, the majority of B cells involved in the production of Abs of a given isotype are specific for the immunizing Ag (a result of essentially exclusive activation by Ag-specific T cells), then the rise seen in total Ig levels would be anticipated to closely parallel Ag-specific levels. In contrast, if class switch and Ig production occur regularly in B cells which are not specific for the immunizing Ag (a result of adjuvant exposure, or due to increases in systemic IL-4 or IFN γ levels), then the increase in total Ig levels would relate poorly to increases in the levels of Abs specific for any one particular Ag.

In the present study, we examined the relationship between total and Ag-specific IgE, IgG1, and IgG2a responses elicited under a wide variety of experimental conditions. We report strong correlations between Ag-specific and total serum Ig for both IgE and IgG1 isotypes, regardless of the phenotype (predominately Th1 versus Th2 biased) or nature (primary versus recall) of the immune response induced in vivo. In contrast, serum lev-

els of Ag-specific and total IgG2a are independent of one another in all types of immune responses examined in C57Bl/6 mice or outbred mice, and exhibit limited correlation in BALB/c mice. These data suggest that increased total IgE and IgG1 levels are chiefly a result of increased levels of Ab with specificity for the immunogen, whereas IgG2a levels reflect broader patterns of B cell activation.

Materials and Methods

Mice

C57Bl/6, BALB/c, CD1 (outbred) and CD8 $^{-/-}$ mice (a kind gift of Dr. T. Mak, University of Toronto) were bred at the University of Manitoba breeding facility (Winnipeg, Canada) or purchased from Charles River Canada (St. Constant, Canada). IL-12 p35 and p40 $^{-/-}$ mice, homozygous on a C57Bl/6 background, were purchased from Jackson Laboratories (Bar Harbor, Me., USA) and bred at the University of Manitoba breeding facility. All mice were housed in a specific-pathogen-free environment and were used in strict accordance with guidelines set by the Canadian Council on Animal Care.

Treatment of Mice

To induce type 2 immune responses, mice were immunized by intraperitoneal injection of 0.2 or 2.0 μ g ovalbumin (OVA) adsorbed onto 2 mg Al(OH) $_3$ (alum) as indicated in table 1. To induce type-1-biased responses, mice were immunized intraperitoneally with 100 μ g OVA in Complete Freund's Adjuvant (CFA) on the days indicated (table 1). In some instances, OVA (alum)-immunized mice were also treated intraperitoneally with 200 ng of IL-12 or 80 μ g of polymerized OVA (OA-POL), both of which are potent inducers of type-1-dominated immunity [19, 20]. The variety of conditions examined was selected to induce a wide range of immune responses, both strong and comparatively weak, thereby providing a more comprehensive picture of the regulation of total and OVA-specific Ig production. Mice were bled 10 and 14 days after initial immunization for determination of primary responses, then 7 and/or 14 days after boost for analysis of recall responses. Serum collected at these time points was used to determine both Ag-specific and total Ig levels.

Determination of Serum Ab Levels

OVA-specific IgE titers were determined by 48-hour passive cutaneous anaphylaxis (PCA) as previously described [21]. Briefly, OVA-specific IgG1 and IgG2a titers were determined by ELISA. OVA-specific IgG was captured on OVA-coated plates while total murine IgG1 and IgG2a were determined using sheep anti-mouse IgG (Southern Biotechnology Associates Inc.) for capture. Detection was carried out using the same biotinylated goat anti-mouse IgG1 or goat anti-mouse IgG2a (Southern Biotechnology Associates Inc.) and streptavidin-conjugated alkaline phosphatase for both OVA-specific and total Ab levels. Color was developed following addition of *p*-nitrophenyl phosphate (Sigma). Pooled immune serum of known OVA-specific IgG1 and IgG2a titer was used as an internal standard. Total IgE was measured by ELISA using rat anti-mouse IgE (Southern Biotechnology Associates Inc., Birmingham, Ala., USA) as a capture antibody followed by biotinylated rat anti-mouse IgE heavy chain mAb (Serotec, UK). Purified anti-DNP mouse IgE prepared from hybridoma 26.82 (a gift of Dr. A. Froese, University of Manito-

ba) was used as a standard. Internal standards of monoclonal mouse IgG1 and IgG2a tissue culture supernatants were calibrated against purified IgG1 mAb generated by Dr. G. Lang (University of Manitoba) and IgG2a (UPC 10, Sigma). For assaying OVA-specific IgG1 in mass units, ascites fluid containing monoclonal mouse anti-OVA IgG1 (MAB2, a gift of Dr. G. Lang) was affinity purified on an OVA-Sepharose-4B column. The concentration of IgG1 in the OVA-specific fraction was determined by ELISA, and subsequently used to determine the concentration of OVA-specific IgG1. The sensitivity of these assays was typically 0.5 ng/ml for IgE, 0.5 ng/ml for IgG1, 0.1 ng/ml for IgG2a and 0.2 ng/ml for OVA-IgG1. The specificity of all assays was confirmed. Reagents specific for one isotype displayed minimal cross-reactivity with other isotypes at 1,000-fold greater concentrations (data not shown).

Statistical Analysis
OVA-specific Ig values were paired with the total Ig levels from the same mouse at the same time point and coefficients of correlation (r) and statistical significance (p) were determined with the least-squares method and subsequent ANOVA analysis using Microsoft Excel 97. p values <0.05 were considered statistically significant.

Results

Induction of Type-1- and Type-2-Dominated Ab Responses
C57Bl/6 mice were immunized with OVA in the presence of CFA or alum in order to induce Th1- or Th2-biased responses, respectively (fig. 1). OVA immunization in the presence of CFA induced high levels of OVA-specific and total IgG2a but low levels of OVA-specific and total IgE, a pattern characteristic of type-1-dominated immune responses. In contrast, immunization of mice with OVA alum induced a type-2-dominated Ab response characterized by elevated IgE levels (OVA-specific and total) and low levels of IgG2a. Certain strains were included that demonstrated substantially different

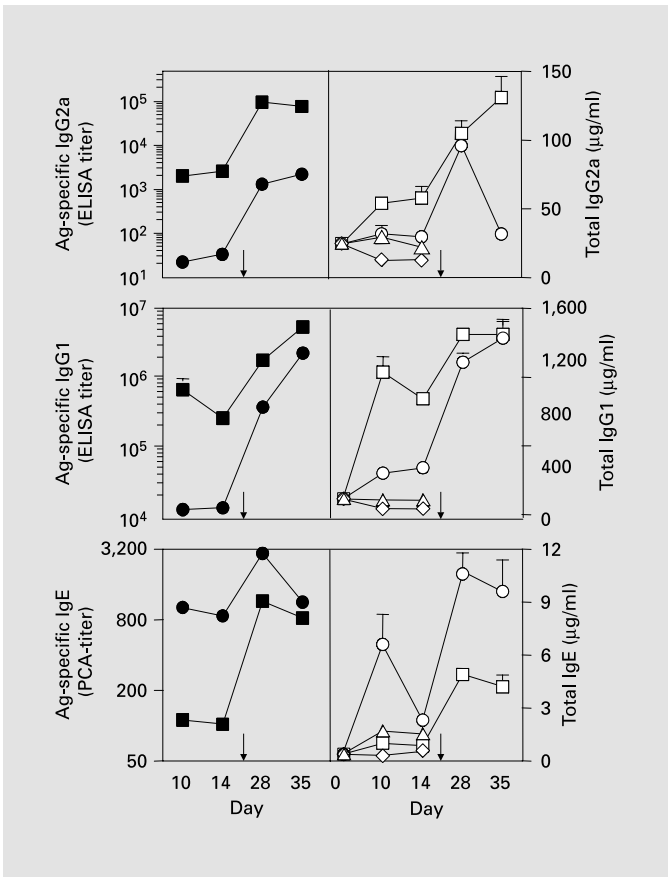


Fig. 1. OVA-specific and total Ig levels in type-1- and type-2-dominated immune responses. C57Bl/6 mice were immunized with OVA in the presence of CFA (squares) or alum (circles) to induce type-1- and type-2-dominated immune responses, respectively. Alternatively, mice were immunized with alum alone (triangles) or CFA alone (diamonds) and bled at days 0, 10 and 14. OVA-specific (open symbols) and total (closed symbols) Ab levels were measured. Arrow on the x-axis indicates time of Ag boost (day 21). Means $\pm$ SEM shown (n = 20 mice per group from a total of 4 experiments), excepting adjuvant only groups which consisted of 4 mice per condition.

Table 1. Immunization protocols, mice strains and treatments used

Dominant immune response induced	Mouse strains used	OVA immunization		Additional treatments		Bleed	
		adjuvant dose, μ g	day	reagent	day	1°	recall
Type 2	C57Bl/6, p35-/-, p40-/-, CD1, BALB/c	alum (2.0)	0, 21/28	none	-	10, 14	28/35, 35
Type 2	C57Bl/6, CD8-/-, CD1	alum (0.2)	0, 76	none	-	none	84
Type 1	C57Bl/6, p35-/-, p40-/-, BALB/c	CFA (100)	0, 21	none	-	10, 14	28, 35
Type 1	C57Bl/6, CD8-/-	alum (0.2)	0, 76	OA-POL	31, 33, 35, 45, 47, 49, 60, 62, 64	NA	84
Type 1	CD1	alum (0.2)	0, 31	IL-12	30, 31, 32, 33, 34	NA	38
Type 1	CD1	alum (2.0)	0, 31	IL-12	30, 31, 32, 33, 34	NA	38

levels of certain Ab isotypes (e.g. ~50% reduced IgG2a levels in IL-12 p35^{-/-} and IL-12 p40^{-/-} mice relative to wild-type controls); however, OVA-specific and total Ab levels were readily detectable in each strain, under all conditions outlined in table 1 (data not shown). Importantly, mice immunized with either adjuvant alone (alum or CFA) did not demonstrate a detectable OVA-specific Ab response or significantly increased total Ig production (fig. 1) and total Ig levels did not increase significantly above the basal (day 0) levels seen in untreated mice (data not shown). Immunization of mice with OVA in the absence of any adjuvant also failed to elicit a detectable Ab response (data not shown). It is interesting to note that total IgG2a levels consistently dropped to levels normally seen in untreated controls by day 35 in OVA (alum)- but not OVA (CFA)-immunized mice, suggesting that alum, as a potent type-2-inducing adjuvant, may not have the same capacity as CFA, an exceptionally potent adjuvant, to trigger sustained elevations in total type 1 Ab production. Thus, Ig production was elicited under a wide variety of conditions (selected to provide both strong and weak type 1 or type 2 immune responses) to provide a more complete analysis of the relationship between OVA-specific and total Ig levels in exogenous Ag-driven responses.

OVA-Specific and Total IgE and IgG1 Production Are Tightly Correlated in OVA (Alum)-Immunized, Outbred Mice

To determine the degree of correlation that exists between Ag-specific and total serum Ig production within IgG1 (as the major serum isotype), IgG2a (as a widely used representative of Th1-dominated immune responses) or IgE responses (as an important marker of Th2 immune responses and allergic sensitization), outbred CD1 mice (reflective of the wide variability seen in genetically diverse populations) were immunized with multiple concentrations of OVA in the presence of alum to induce

specific antibody responses ranging over 3–4 orders of magnitude (i.e. anti-OVA IgG2a ELISA titers of 20 to 10⁵). As reported in table 2, regardless of the amount of OVA used for immunization, OVA-specific and total IgE were consistently and highly correlated ($r > 0.9$; $p < 0.00001$). A correlation between OVA-specific and total IgG1 was evident, but substantially less so, with correlation coefficients of $r = 0.64$ to 0.81 (table 2). In contrast to the strong correlations seen with OVA-specific and total IgE or IgG1, IgG2a responses in CD1 mice were consistently independent of one another (table 2). Thus, analysis of IgE and IgG1 responses indicated that OVA-specific titers are tightly correlated with total serum Ig levels in CD1 mice undergoing strong type 2 immune responses.

Ag-Specific and Total IgE and IgG1 Are Consistently and Strongly Correlated during Type 2 Immune Responses

The relationships between OVA-specific and total IgE, IgG1 and IgG2a were next examined in the two most widely used inbred strains, BALB/c and C57Bl/6. Since Ig synthesis is often measured in models of disease states where altered Ab responses are evident, the relationship between total and OVA-specific Ab responses in various knockout strains was also examined. The strains used include CD8^{-/-} (which have decreased IgE levels – unpublished observation), and both IL-12 p35^{-/-} and p40^{-/-} mice (which have substantially decreased IgG2a synthesis [21, 22]) all of which are on a C57Bl/6 background.

As displayed in table 3, OVA-specific and total IgE levels are consistently correlated in mice undergoing type-2-dominated immune responses, with correlation coefficients of up to 0.971. As seen for OVA (alum)-immunized CD1 mice, Ag specific and total IgG1 levels also demonstrated a high degree of correlation, with r values generally >0.5 (table 3). In marked contrast, OVA-specific and total

Table 2. OVA-specific and total IgE and IgG1 are highly correlated in outbred CD1 mice undergoing type 2 immune responses^a

Strain	n/x ^b	OVA dose μg	IgE		IgG2a		IgG1	
			r	p	r	p	r	p
CD1	11.3	0.2	0.921	5.6 × 10⁻⁵	0.458	0.18	0.639	0.034
CD1	9.3	2	0.976	7.0 × 10⁻⁶	0.434	0.24	0.814	0.0075

^a Significant correlations ($p < 0.05$) between OVA-specific and total Ig levels are indicated in bold.

^b n/x indicates n mice in x separate experiments.

IgG2a were weakly correlated or not correlated. Interestingly, the exception was BALB/c mice in which $r = 0.614$ (table 3). Thus, in inbred strains of mice, both OVA-specific and total IgE and IgG1 levels are highly correlated during type-2-biased immune responses. These data also demonstrate that in situations where genetic manipulation results in markedly different Ab responses, significant correlations between OVA-specific and total IgE and IgG1 remain similarly evident.

Total and OVA-Specific IgE and IgG1 Levels Are Highly Correlated following Immunization under Type-1-Inducing Conditions

Relationships between OVA-specific and total Ig production were next analyzed under a variety of type-1-inducing conditions including immunization with OVA in the presence of CFA or immunization with OVA combined with rIL-12 or OA-POL treatment, following protocols previously shown to potently elicit type-1-oriented antibody responses [19, 20]. As shown in table 3, in both C57Bl/6 and BALB/c mice undergoing such responses, the correlation between Ag-specific and total IgE levels was extremely strong, with correlation coefficients of 0.7–0.8. Mirroring what occurs under type-2-dominated conditions, specific and total IgG1 synthesis were also highly correlated, with correlation coefficients of $r = 0.6$ –0.8 (table 3). It is interesting that despite much higher levels of IgG2a production in mice immunized under type-1-inducing conditions than those seen under type 2 conditions (OVA-specific IgG2a titers ranged from ~1,400 to 86,000 and total IgG2a levels ranged from 9 to 270 $\mu\text{g/ml}$; fig. 1), only BALB/c mice display consistent correlations

between OVA-specific and total IgG2a levels. In each of the genetically manipulated strains immunized under type-1-inducing conditions, levels of OVA-specific and total IgE and IgG1 were again consistently and strongly correlated, while the relationship between OVA-specific and total IgG2a was weak and inconsistent (data not shown). Similar patterns of correlation were seen when each type-1-inducing immunization regimen was assessed independently (data not shown). Thus, in IgG2a-dominated, type 1 immune responses, OVA-specific and total IgE and IgG1 are highly correlated as in type-2-dominated immune responses.

OVA-Specific and Total IgE and IgG1 Are Tightly Correlated Regardless of the Type and Intensity of Immune Response

Antibody responses induced following reexposure to exogenous Ag are much more rapid and intense, with a substantially different balance of isotypes than seen during primary responses. To determine if the correlations between OVA-specific and total IgE and IgG1 were similarly evident in both primary and recall immune responses, data were compared in terms of the nature of immune response examined (primary versus recall). As demonstrated in table 4, the relationship between OVA-specific and total IgE or IgG1 were strong in both primary and recall responses. As before, OVA-specific and total IgG2a levels were independent of one another ($r < 0.1$) (table 4).

Finally, to obtain a global picture of the relationship between OVA-specific and total Ig synthesis, all data collected, regardless of phenotype (type 1 versus type 2), nature (primary versus recall) or genetic background were

Table 3. Ab correlations in various strains undergoing OVA-specific immune responses^a

Strain	Type of response	n/x ^b	IgE		IgG2a		IgG1	
			r	p	r	p	r	p
C57Bl/6	type 2	50/3	0.662	9.2×10^{-5}	0.053	0.692	0.630	4.2×10^{-8}
BALB/c	type 2	30/3	0.566	0.0011	0.614	0.0003	0.841	5.6×10^{-9}
p35-/- ^c	type 2	54/4	0.971	3.3×10^{-20}	0.169	0.183	0.357	0.003
p40-/-	type 2	46/3	0.619	0.0006	0.545	0.108	0.663	2.6×10^{-8}
CD8-/-	type 2	9/3	0.816	0.0074	0.294	0.520	0.725	0.027
C57Bl/6	type 1	54/3	0.685	5.6×10^{-8}	0.190	0.130	0.593	5.7×10^{-6}
BALB/c	type 1	31/3	0.846	2.1×10^{-9}	0.865	3.6×10^{-10}	0.847	1.8×10^{-9}

^a Significant correlations ($p < 0.05$) between OVA-specific and total Ig levels are indicated in bold.

^b n/x indicates n mice in x separate experiments.

^c All knockout mice are on a C57Bl/6 background.

compiled and correlation coefficients determined (fig. 2). OVA-specific and total IgE or IgG1 levels were highly correlated ($r = 0.696$ and $r = 0.605$, respectively). In contrast, levels of total IgG2a were poorly predictive of OVA-specific IgG2a levels, a fact reflected in the scatter plot of OVA-specific versus total IgG2a ($r = 0.197$) (fig. 2). Interestingly, OVA-specific and total IgE and IgG1 responses from mice undergoing both type-1- and type-2-dominated immune responses were highly correlated. This confirms that the correlation between specific and total IgE or IgG1 is independent of the phenotype of the induced antibody response.

Similar Increases in Absolute Amounts of Total and OVA-Specific IgG1 following Immunization

The strong correlation that exists between total and Ag-specific IgE/IgG1 suggests that the increases in total Ig levels are chiefly a result of increases in OVA-specific Ig production. To test this hypothesis, we assessed the absolute increases in the mass of both total and OVA-specific IgG1 levels in the serum following immunization with OVA (alum) or OVA (CFA). As shown in figure 3, following immunization under type-1- and type-2-inducing conditions, absolute increases in OVA-specific IgG1 were sufficient to account for the observed increases in total IgG1 (fig. 3). These data further indicate that increases in total IgG1 are chiefly a result of increased OVA-specific IgG1 production.

Discussion

Ig isotype class switch and induction of antibody production is a complex process which requires delivery of multiple signals to B cells. Class switch is induced by IL-4 or IL-13 triggered binding of phosphorylated Stat6- to Stat6-binding elements in the promoter regions of IgE [7] and IgG1 [8] genes. Recent evidence indicates that once class switch has occurred, ligation of CD86 on the B cell

surface results in increased production of both IgE and IgG1 [11]. Although IgG1 has been frequently used as a marker of type 2 immunity, primarily because of the ease with which it is measured relative to the less abundant IgE, it has been known for over a decade that it is regulated independently of IgE [2, 5, 23, 24], strongly arguing that IgE is the more appropriate marker for the intensity of type 2 immune responses. In contrast, while class switch to IgG2a is driven by IFN γ [14], it is not subject to upregulation following signaling through CD86 [11]. NK cells are a significant source of IFN γ in vivo after infection with various pathogens [25–27] or administration of IL-12 [19]. Selective activation of NK cell populations was found to enhance Ag-specific IgG2a responses [15, 18].

The novel finding that there exists a strong correlation between OVA-specific and total IgE levels (and to lesser extent OVA-specific and total IgG1 levels) suggests that increases in total IgE/IgG1 levels are chiefly a result of increased levels of OVA-specific Abs – a hypothesis which is supported by the finding the majority of the increase in total IgG1 production is accounted for by the increase in OVA-specific IgG1. This finding is also consistent with the strong T cell dependence of the signals involved in IgE/IgG1 induction and expression (i.e. IL-4, CD40 and CD86). In contrast, the lack of a consistent correlation between OVA-specific and total IgG2a levels, even in the midst of a Th1-, IgG2a-dominated immune response (fig. 2, 3), suggests that substantial levels of non-OVA-specific IgG2a are being produced. This may be a result of nonspecific activation of NK cells (previously found to increase IgG2a levels [15, 18]), or activation of other cell types.

It is interesting that there is a consistent correlation between total and Ag-specific IgG2a production in BALB/c mice. It has been demonstrated that macrophages from BALB/c produce less IL-12 following LPS stimulation compared to the great majority of other strains of mice (including C57Bl/6, C3H, DBA, AKR, CBA and A/J mice) [28], suggesting that BALB/c may be unique in this

Table 4. Correlation of OVA-specific and total Ab correlations in primary and recall immune responses^a

Type of response	n	IgE		IgG2a		IgG1	
		r	p	r	p	r	p
Primary	200	0.803	3.0×10^{-35}	0.086	0.192	0.44	1.23×10^{-11}
Recall	275	0.615	1.5×10^{-27}	0.061	0.296	0.523	3.5×10^{-21}

^a Significant correlations ($p < 0.05$) between OVA-specific and total Ig levels are indicated in bold.

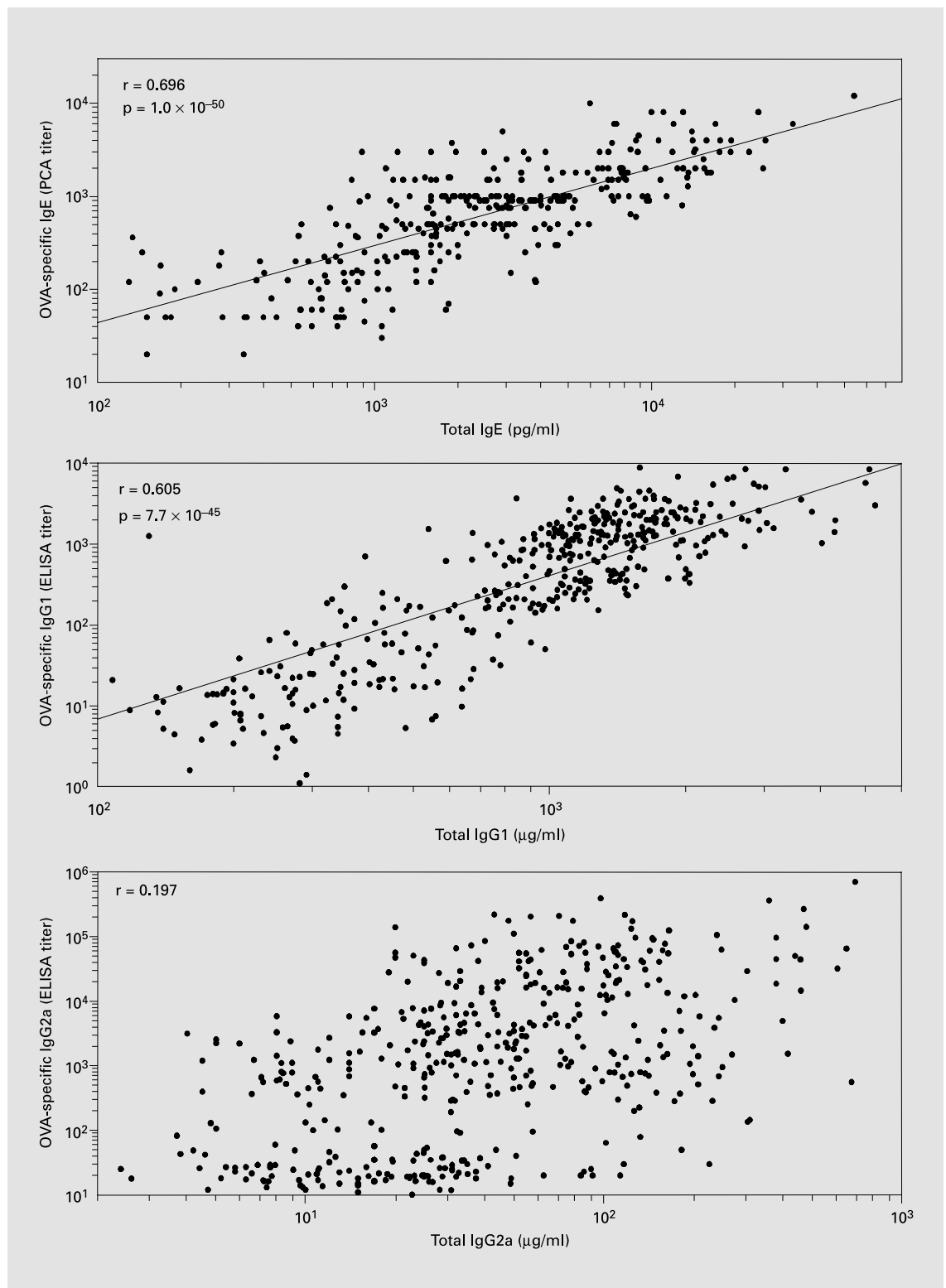


Fig. 2. Total and OVA-specific IgE and IgG1 levels are highly correlated in OVA immunized mice regardless of strain or method of immunization. C57Bl/6, BALB/c, IL-12 p35 $^{-/-}$, IL-12 p40 $^{-/-}$, and CD8 $^{-/-}$ mice were immunized with OVA (alum) or OVA (CFA) and treated with nothing, IL-12 or OA-POL as indicated in table 1. Each point on the scatter plot represents a single mouse at a single time point (n = 475 points).

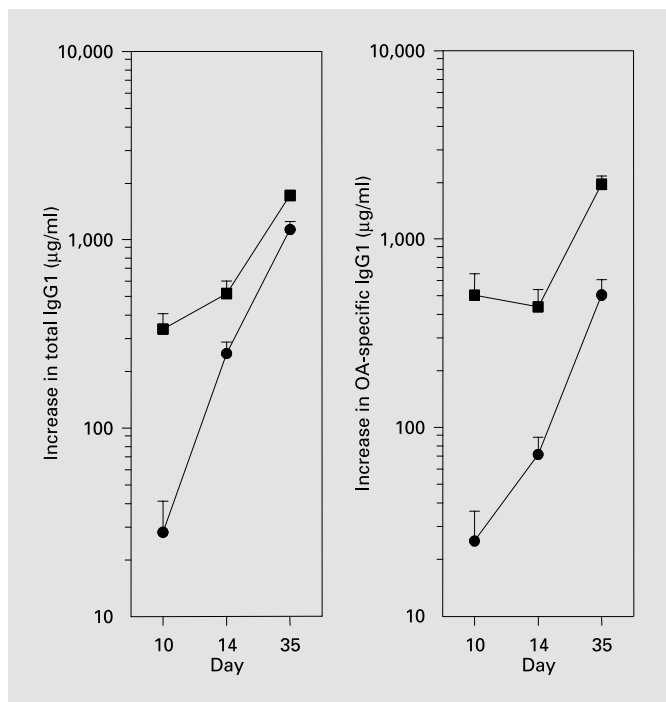


Fig. 3. Increases in total IgG1 attributable to increases in OVA-specific IgG1. C57Bl/6 mice were immunized with OVA (CFA) (circles) or OVA (alum) (squares) to induce type-1- or type-2-biased responses, respectively. The concentration of OVA-specific IgG1 and the increase in total IgG1 levels (above pre-immunization levels obtained for each mouse – ranging from 71 to 390 μg/ml) were measured. Means ± SEM shown (n = 12 mice from 3 experiments).

respect. Thus, knockouts on a BALB/c background may exhibit different responses from the mice studied above. In part, this may be due to the fact that BALB/c mice also produce less IFN γ in response to IL-12 stimulation due to decreased IL-12R and STAT4 expression [29, 30]. Thus, we hypothesize that due to the deficiency in IL-12/IL-18-driven IFN γ production seen in BALB/c there is a global decrease in nonspecific IFN γ production following immunization with Ag and adjuvant. This deficiency in IFN γ production in turn leads to a limited induction of Ag-nonspecific IgG2a. In contrast, in mice with intact IL-12/IL-18-driven IFN γ production (most other inbred and outbred strains), there is significant induction of nonspecific IFN γ production which enhances total IgG2a levels, resulting in the poor association between total and Ag-specific IgG2a levels seen in these mice.

These data provide an interesting contrast to human antibody responses where total and specific IgE levels are often judged to be independent, in that Ag-specific but not total IgE levels associate with immediate hypersensi-

tivity. Whether this is a consequence of relatively imprecise units of measurement used clinically (i.e. conversion of ImmunoCAP results in an arbitrary, less precise 'Class 0 to 6' ranking scheme [31, 32]) or reflects an inherent difference in human versus mouse biology remains to be determined.

In animal models used for study of human diseases, many only examine the primary antibody response to conserve time and minimize the requirements for numerous injections [33–35]. The more relevant situation, in that it is the norm in disease, is the recall antibody response. We found that OVA-specific and total IgE levels, and to a lesser extent IgG1 levels, were highly correlated in both primary and recall immune responses. In contrast, specific levels and total IgG2a levels are usually independent of one another. This suggests that the mechanisms by which total and Ag-specific Ab production is regulated are similar in primary and recall antibody responses. This hypothesis is supported by regression analysis performed on plots of OVA-specific versus total Ig levels which reveal similar slopes for primary and secondary IgE or IgG1 responses (data not shown). In contrast, regression analysis of OVA-specific and total IgG2a levels (data not shown) revealed significantly different slopes in primary and recall immune responses.

In summary, this study demonstrates that levels of Ag-specific IgE and total IgE are strongly correlated in a wide variety of murine strains, utilizing six different immunization protocols which elicit IgE responses spanning 4 orders of magnitude. Ag-specific and total IgG1 exhibit similar, albeit less robust, correlations, suggesting that increases in total IgE or IgG1 levels are primarily a result of increases in immunogen-specific levels of these isotypes. In BALB/c mice, which have a demonstrated deficiency in IFN γ production, there is a correlation between total and Ag-specific IgG2a. However, in mice with optimal IFN γ production, the same strong relationship does not exist between OVA-specific and total IgG2a levels, arguing that during both developing and recall Ab responses, there is substantial production of IgG2a which are not specific for the immunogen.

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