Clinical & Experimental Immunology
The Journal of Translational Immunology
doi:10.1111/cei.12824 REVIEW SERIES: AGEING AND THE IMMUNE SYSTEM, EFFECTS OF IMMUNOSENESCENCE AND CLINICAL IMPLICATIONS
Clinical and Experimental Immunology REVIEW ARTICLE Series Editors: Birgit Weinberger and Arne Akbar

Ageing and obesity similarly impair antibody responses

OTHER ARTICLES PUBLISHED IN THIS REVIEW SERIES
Immunosenescence: the importance of considering age in health and disease. Clinical and Experimental Immunology 2017, 187: 1–3.
The convergence of senescence and nutrient sensing during lymphocyte ageing. Clinical and Experimental Immunology 2017, 187: 4–5.
Immune senescence: significance of the stromal microenvironment. Clinical and Experimental Immunology 2017, 187: 6–15.
Innate immune responses in the ageing lung. Clinical and Experimental Immunology 2017, 187: 16–25.
Age-related alterations in immune responses to West Nile virus infection. Clinical and Experimental Immunology 2017, 187: 26–34.
Intracellular signalling pathways: targets to reverse immunosenescence. Clinical and Experimental Immunology 2017, 187: 35–43.
Ageing and inflammation in patients with HIV infection. Clinical and Experimental Immunology 2017, 187: 44–52.
Considerations for successful cancer immunotherapy in aged hosts. Clinical and Experimental Immunology 2017, 187: 53–63.
The life cycle of a T cell after vaccination – where does immune ageing strike? Clinical and Experimental Immunology 2017, 187: 71–81.
Herpes zoster and the search for an effective vaccine. Clinical and Experimental Immunology 2017, 187: 82–92.
Adult vaccination against tetanus and diphtheria: the European perspective. Clinical and Experimental Immunology 2017, 187: 93–99.

D. Frasca, A. Diaz, M. Romero and B. B. Blomberg
Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, USA

Accepted for publication 8 June 2016
Correspondence: D. Frasca, Department of Microbiology and Immunology, University of Miami Miller School of Medicine, PO Box 016960 (R-138), Miami, FL 33101, USA.
E-mail: dfrasca@med.miami.edu

Summary

Ageing is characterized by increased low-grade chronic inflammation, which is a significant risk factor for morbidity and mortality of elderly individuals. Similar to ageing, obesity is considered to be an inflammatory predisposition associated with chronic activation of immune cells and consequent local and systemic inflammation. Both ageing and obesity are characterized by reduced innate and adaptive immune responses. This review focuses on B cells, how they may contribute, at least locally, to low-grade chronic inflammation in ageing and obesity and on the mechanisms involved.

Keywords: aging, B cells, inflammation, obesity

Ageing and related inflammation decrease B cell responses

With ageing, the innate and adaptive immune responses deteriorate, leading to greater susceptibility to infectious diseases and reduced responses to vaccination [1]. The decreased ability of aged individuals to respond effectively against infectious agents and vaccines includes defects in T cell signalling to B cells [2–4], reduced somatic hypermutation (SHM) [5,6] and class-switch recombination (CSR) in germinal centre B cells [7], and intrinsic shifts in the V_H repertoire [8]. Functional alterations in T cells have been considered for a long time to be sufficient *per se* to explain the age-related decrease in antibody responses to exogenous antigens and vaccines in elderly people. However, a large amount of work has been conducted more recently showing that defects in other components of the innate and adaptive immune systems also occur with age and contribute to the increased frequency and severity of infectious diseases in elderly people.

Our laboratory has characterized age-related intrinsic B cell defects, which are responsible for suboptimal antibody responses of elderly individuals to infections and vaccines [9–13]. A reduction in activation-induced cytidine deaminase (AID), the enzyme necessary for CSR, SHM and immunoglobulin (Ig)G production, as well as in E47, a key transcription factor regulating AID [14], has been identified by our group. It has also been established that AID correlates with optimal B cell function and therefore AID can be used a predictive marker of optimal B cell response. The decrease in AID and E47 leads to a reduced ability to generate higher-affinity vaccine-specific antibodies [5]. The antibody response to the seasonal and pandemic influenza vaccines measured in serum is associated with the B cell response after vaccination to the vaccine *in vitro*. *In-vivo* and *in-vitro* B cell responses have been measured, respectively, by the haemagglutination inhibition assay and by AID mRNA expression by quantitative polymerase chain reaction (qPCR) after B cell restimulation with the vaccine. It has been shown that the specific response of B cells to vaccination *in vivo* and *in vitro* are both decreased by ageing and are correlated significantly [5,11,13]. Moreover, the percentages of switched-memory B cells and cytosine–phosphate–guanine (CpG)-induced AID before vaccination are both good B cell biomarkers that are reduced in elderly people and are correlated significantly with the *in-vivo* antibody response to the vaccine [11,13], indicating that they can be used as predictive biomarkers of optimal vaccine-induced antibody responses.

It has been shown that elderly individuals have a significant reduction in B cell repertoire diversity and that this correlates with their health status, and that B cell clonal expansions with age had been reported previously [15].

Using high-throughput long read sequencing of human antibody repertoires in the context of Epstein–Barr virus (EBV) or cytomegalovirus (CMV) serum positivity [16] and influenza vaccination [17], it has been shown that elderly individuals have decreased numbers of lineages but increased prevaccination mutation load in their repertoire and the diversity of the lineages is reduced greatly compared to young individuals, consistent with earlier reports on contraction of B cell repertoires in elderly people [18]. These findings could help to explain the impaired vaccine responses observed in elderly people.

The inflammatory status of an individual may impact upon the function of cells of the immune system. B cells from elderly individuals spontaneously make higher amounts of tumor necrosis factor (TNF)-α than those from young subjects and B cell intrinsic TNF-α levels are correlated positively with serum TNF-α. Importantly, these B cell levels of TNF-α before stimulation are correlated negatively with the response of the B cells from the same individual after *in-vitro* stimulation which is measured by AID [9]. Additionally, high prevaccine serum and B cell TNF-α levels are also correlated negatively with the *in-vivo* serum response to the influenza vaccine [9]. In line with these results, an anti-TNF-α antibody was found to increase significantly the response in cultured B cells from elderly individuals, providing a proof-of-principle that it is possible to improve antibody production in elderly individuals by counteracting autocrine TNF-α [9]. These findings identify serum and cytoplasmic B cell TNF-α as other B cell-specific biomarkers, which can help to predict the quality of *in-vivo* and *in-vitro* B cell responses. Although our studies have shown that serum and B cell TNF-α are correlated positively in the majority of individuals, some people show lower levels of B cell TNF-α and lower AID. These results suggest that not only TNF-α but also other markers of intrinsic B cell inflammation may contribute to the down-regulation of AID in B cells from aged individuals; for example, microRNAs, which have been correlated negatively with AID [19].

Ageing is characterized not only by increased circulating levels of proinflammatory cytokines [TNF-α, interleukin (IL)−6, C-reactive protein (CRP)], but also by latent infections with viruses such as CMV. Stimuli triggering inflammation can also be generated by the age-related increase in the amount of self-debris due to the continuous turnover of cells and tissues, such as circulating mitochondrial DNA (mtDNA) and post-translationally modified macromolecules (DNA or proteins modified by oxidation, acylation, glycosylation), which are recognized by immune sensors as exogenous and represent a potent inflammatory stimulus [20].

CMV-seropositivity has been shown to have a negative effect on influenza vaccine-specific antibody responses. Our group has demonstrated recently for [21] the first time a negative association between CMV seropositivity and the B cell predictive biomarkers of optimal vaccine responses characterized previously in our laboratory, and found CMV seropositivity associated with increased levels of serum and B cell-intrinsic TNF-α; this increase in inflammation, contributed in part by CMV, may be one of the mechanisms to down-regulate the B cell antibody response. One proposed mechanism by which CMV decreases B cell function may be an increase in systemic/serum TNF-α which induces B cell-derived TNF-α which, in turn, activates the promoter/enhancer of CMV and proinflammatory cytokine production. In addition to this mechanism acting directly on B cells, CMV may also down-regulate the antibody response to the influenza vaccine indirectly through the induction of terminally differentiated T cells and accumulation of senescent T cells [22,23], which could lead to reduced generation of memory T cells [24,25].

Obesity is associated with decreased B cell responses

Obesity and obesity-related diseases are a significant risk to public health, and the numbers of obese individuals in the United States have increased dramatically in the last few years (Obesity Data Prevalence Map, http://www.cdc.gov/obesity/data/prevalence-maps.html). More than one-third of US adults are obese, with more in the African American population, and these numbers are predicted to increase alarmingly in the next few years. Increased weight and abnormal accumulation of fat tissue lead to detrimental health consequences, mainly because increased adipose tissue is associated with increased inflammation, insulin resistance (IR), p53 activation and telomere shortening [26,27]. The growing interest in the field of obesity research is aiming to discover and unravel the cellular and molecular mechanisms leading to the increase in body weight and how this affects health outcomes. While prevention is of great importance, it is medically relevant to identify biological pathways with the potential to treat obesity and related disorders, particularly in adults with fully established obesity and associated conditions.

Obesity is associated with chronic activation of cells of the innate immune system and consequent local and systemic inflammation, which contributes to pathological conditions such as type 2 diabetes (T2D) [28–30], cancer [31], psoriasis [32], atherosclerosis [33] and inflammatory bowel disease [34]. Obesity is linked to increased susceptibility to bacterial, viral and fungal infections [35,36], and obese individuals develop more postsurgical infections than do lean individuals [37,38].

The adipose tissue is a major immunologically active organ that contributes to systemic inflammation. Adipose tissue inflammation is characterized by infiltration and activation of immune cells that produce cytokines and chemokines that contribute to the ongoing chronic inflammation that promotes the degradation of metabolic pathways

Table 1. Effect of obesity and ageing on B cell function in humans

	Individuals			
	Young Lean	Young Obese	Elderly lean	Elderly Obese
In vivo influenza vaccine response[a]	196 ± 33	80 ± 16**	56 ± 8^^^	28 ± 4** ^^
B cell subsets in blood[b]				
– Switched Memory	15 ± 0.9	6 ± 0.6****	4 ± 0.5^^^^	2 ± 0.3** ^^^^
– IgM Memory	29 ± 1	27 ± 2	22 ± 2	21 ± 3
– Naïve	50 ± 0.8	57 ± 2**	61 ± 2^^^	64 ± 0.9^^
– Late/Exhausted Memory	4 ± 0.4	12±i ****	12 ± 0.6^^^^	14 ± 2
– Transitional B cells	8 ± 0.9	4 ± 0.5**	3 ± 0.6^^^	1.7 ± 0.4* ^^
Cytokine production				
– icTNF-a in unstimulated B cells[c]	5 ± 3	18 ± 2*	14 ± 2^	26 ± 1*** ^^
– IL-6 in stimulated B cells[d]	68 ± 6	244 ± 24****	181 ± 20^^^	323 ± 2o** ^
– IL-10 in stimulated B cells[d]	58 ± 3	37 ± 2**	26 ± 4^^^^	6 ± 0 6*** ^^^^
CSR				
– AID expression in stimulated B cells[e]	0.2 ± 0.03	0.09 ± 0.01*	0.08 ± 0.01^^	0.03 ± 0.003* ^^^
– E47 expression in stimulated B cells[e]	0.16 ± 0.008	0.08 ± 0.008***	0.06 ± 0.007^^^^	0.03 ± 0.004* ^^^
– IgG in sups of stimulated B cells[d]	41 ± 4	2o±3***	21 ± 2^^	7 ± 1*** ^^

Results are from reference 48 (Frasca et al., Obesity 2016).

[a]Results are reciprocal of the titers after vaccination.

[b]Results are percentages of CD19+ B cells.

[c]Results are percentages of icTNF-a-positive B cells, as evaluated by flow cytometry and intracellular (ic) staining.

[d]Measured by ELISA.

[e]Measured by qPCR.

*$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$ indicate significant differences between lean and obese within the same age group.

^$p<0.05$, ^^$p<0.01$, ^^^$p<0.001$, ^^^^$p<0.0001$ indicate significant differences between young and elderly within the same weight group.

in obesity. Most of the studies conducted so far support a crucial role for proinflammatory T cells and macrophages in promoting local inflammation in the visceral adipose tissue (VAT) leading to IR. It has been shown that, in obesity, IFN-γ-producing $CD8^+$ and T helper type 1 (Th1) $CD4^+$ T cells infiltrate VAT [39] and promote secretion of proinflammatory cytokines from M1 macrophages which contribute to both local and systemic IR [40]. Conversely, in lean individuals, IL-4/5/13-producing Th2 $CD4^+$ T cells, $CD4^+$ regulatory T (T_{regs}) and invariant natural killer (iNKT) cells are predominant in the VAT and promote secretion of IL-10 and other anti-inflammatory cytokines from M2 macrophages which maintain insulin sensitivity (IS). There is increasing evidence that subcutaneous adipose tissue (SAT) in the belly may also be dangerous in promoting inflammation [41].

Studies elucidating B cell function in obesity are limited, although B cells have emerged recently as crucial players in regulating inflammation in murine VAT by presenting antigens to T cells, secreting proinflammatory cytokines and secreting pathogenic antibodies [42]. B cells infiltrate the expanding adipose tissue in response to hypernutrition [43]. B cells can be activated by products of altered lipolysis in the expanding adipose tissue to release proinflammatory cytokines (TNF-α/IL-6) or chemokines (IL-8), thus contributing to local and systemic inflammation [44,45]. Antibodies can also regulate obesity at the level of lipid absorption from the gut and B^{null} mice show reduction in lipid absorption [46]. Consistently, B^{null} mice fed with a high-fat diet show reduced visceral fat pad weights compared to wild-type controls, suggesting that B cells play a role in changing nutrient absorption, perhaps through local inflammatory responses which could also shape mucosal immunity and change gut microbiota [42,47]. Moreover, murine and human B cells support T cell inflammation in obesity [48].

Our group has shown recently [49] that obesity is associated with attenuated *in-vivo* and *in-vitro* antibody responses in both young and elderly individuals and that the peripheral B cell pool of individuals with obesity is characterized by decreased percentages of anti-inflammatory B cell subsets (transitional B cells) and increased percentages of proinflammatory late/exhausted memory B cells. Moreover, total B cells from both young and elderly individuals with obesity, compared to lean individuals, have impaired function, as measured by AID in response to CpG stimulation, and they secrete more proinflammatory (IL-6) and fewer anti-inflammatory (IL-10) cytokines in culture supernatants. Before stimulation, total

B cells from obese individuals show higher immune activation (IA), as measured by increased levels of intracellular TNF-α (icTNF-α), TLRs and inflammatory micro-RNAs (miRs), all of which associate negatively with AID in stimulated B cells. B cells from young and elderly individuals with obesity also support the production of the proinflammatory cytokines IL-17 and IFN-γ in T cells. These results are summarized in Table 1. In conclusion, the effects of ageing seem to be more pronounced than those of obesity on *in-vivo* responses to the influenza vaccine and on circulating B cell subsets. Conversely, obesity, more than ageing, seems to affect the induction of IA markers in unstimulated B cells (higher icTNF-α and IL-6 in young obese *versus* lean individuals and lower IL-10 in elderly obese *versus* lean individuals). Also, CSR seems to be affected more by obesity in elderly individuals, although values are already very low in the lean ones.

Mechanisms for the down-regulation of B cell responses in ageing and obesity

As summarized above, ageing and obesity are associated with metabolic, physiological and functional changes. Both ageing and the increase in fat mass lead to higher production of proinflammatory cytokines and chemokines, which increase the risk of developing chronic diseases and decrease life expectancy. Moreover, glucose tolerance decreases with age [50] and percentages of abdominal (visceral and subcutaneous) fat also seem to be the major determinants of IR in elderly individuals. Because of these similarities, it is likely that these conditions share similar cellular pathways.

In humans, adiposity increases with age. Computational tomography scans have shown that as age increases SAT decreases, whereas VAT increases [51]. Aged mice also develop increased fat mass, with an increase in VAT, similar to elderly humans [52]. In humans and mice, VAT and SAT are biologically distinct in terms of secretion of proinflammatory mediators, including leptin, the adipocyte-derived factor linking nutritional status with neuroendocrine and immune functions, with VAT being more inflammatory. Furthermore, expression of adipokines from adipose tissue is regulated by nutrients, and these responses are increased with ageing [53].

One of the mechanisms responsible for reduced B cell function in individuals with advanced age or obesity may be leptin-induced systemic and B cell intrinsic inflammation. Plasma levels of leptin, produced by fat cells, correlate with the amount of body fat and body mass index (BMI) and increase with age [54–56]. High serum levels of leptin contribute to the inflammatory state of the adipose tissue associated with obesity [57,58]. Leptin can modulate both innate and adaptive immune responses. For example, it regulates the macrophages' acute inflammatory response by inducing the secretion of proinflammatory cytokines [59,60], activates B cells to produce cytokines [61], promotes B cell survival by inhibiting apoptosis and inducing cell cycle entry [62], controls the activation of $CD4^+$ effector T cells [63] and regulates the balance between proliferation and anergy in T_{regs} [64]. *In-vitro* incubation of B cells from lean individuals with leptin increases phospho-signal tranducer and activation of transcription factor (STAT)-3, crucial for TNF-α production, and decreased phospho-AMP-activated protein kinase (AMPK), the energy-sensing enzyme upstream of AID activation [49].

Another mechanism by which the adipose tissue may down-regulate B cell function and induce the phenotypical and functional changes in B cell subsets observed in individuals with obesity is the secretion by the adipocytes of proinflammatory mediators contributing to systemic chronic inflammation, and of chemokines promoting the migration of B cells to the VAT and regulating B cell function in the VAT. Experiments in progress in our laboratory are investigating these hypotheses.

Telomeres are key markers of biological ageing [65,66] and are predicitve biomarkers of effective B cell responses to the influenza vaccine [67]. In particular, B cells from individuals with protective titres to the influenza vaccine have significantly longer telomeres than those with poor antibody responses [67]. The age-related increase in inflammation decreases telomere length. Shorter telomeres have also been associated with increased BMI and increased waist : hip ratio and VAT accumulation [68], suggesting that obesity may accelerate the ageing process.

Therapeutic interventions to reduce obesity and delay ageing

Although recent studies have shown that overweight and mildly obese elderly individuals may live longer than their normal weight controls, obese elderly individuals may live a greater portion of their life with some disability [69,70]. With the increasing obese population worldwide and with the increasing elderly obese population [71], the development of safe and effective therapeutic interventions is needed urgently. These will not only enhance immune responses to infectious agents and vaccines, but will also improve the biological quality of life in these individuals.

Several epidemiological studies have evaluated the effects of different types of diet in protecting subjects from diseases associated with chronic low-grade inflammation. Traditional diets (Mediterranean) and other less-known diets (Okinawan, DASH, Portfolio) share similar dietary patterns, all of which are associated with reduced risk for inflammation-associated diseases. The primary goal is to treat obese patients to reduce their weight, and this would also correct their other co-morbidities, as not all obese individuals will have and/or take the option of bariatric surgery. Calorie or food restriction without malnutrition has been shown to delay ageing in animal models [72].

This intervention prolongs the lifespan of different animal species, from single-cell organisms to mammals, and prevents or delays age-related diseases such as cardiovascular disease [73], cancer [74] and renal failure [75]. In humans, short-term calorie restriction decreases the expression of biomarkers of reduced longevity, such as fasting insulin, glucose levels and body temperature [76], similar to what has been observed in mice [72].

Obesity is associated with low circulating levels of 25-hydroxyvitamin D [25(OH)D], VitD [77], which decrease the development of obesity-related pathophysiological disorders such as adipose tissue inflammation and subsequent IR. Using high-throughput methodology (transcriptomics), it has been shown that VitD reduces chemokine expression by adipocytes and macrophage migration *in vitro* and limits NF-κB activation [78]. By limiting inflammation, VitD supplementation has been proposed to also benefit frail elderly people by enhancing long-term health [79].

Pharmacological approaches are also being considered, and these include inhibitors of glycolysis, growth hormone/insulin-like growth factor 1 (GH/IGF-1) and inflammatory pathways, as well as activators of the AMPK pathway and treatments with metformin, statins and β-blockers [80]. Some of these interventions have already been shown to improve immune responses in elderly individuals. As an example, a recent paper has shown that pharmacological inhibition of mTOR with the mTOR inhibitor RAD0 increased significantly the *in-vivo* response to the influenza vaccine in elderly healthy individuals, and reduced the percentage of CD4 and CD8 T lymphocytes expressing the programmed death-1 (PD-1) receptor, which inhibits T cell signalling and is expressed more highly with age [81].

In conclusion, autonomous B cell biomarkers of ageing and inflammation, which affect the production of protective antibodies, have been identified and characterized. Investigation of the mechanisms whereby inflammation and immune activation disrupt a functional immune response adds a novel dimension to the current focus on the relationship of inflammation with long-term metabolic disease outcome. These studies will allow targets for design of possible adjuvants, new drugs and/or non-invasive lifestyle changes to improve the immune and effective vaccine responses.

Acknowledgements

This study was supported by NIH AI096446, AG042826 and AG032576 (B. B. B. and D. F.). We thank the volunteers who participated in this study, the personnel of the Department of Family Medicine and Common Health at the University of Miami Miller School of Medicine, in particular Dr Robert Schwartz, chairman for the recruitment of healthy volunteers, and the SCCC Flow Cytometry Core Resource.

Disclosure

No potential disclosures relevant to this paper are reported.

References

1 Boraschi D, Del Giudice G, Dutel C, Ivanoff B, Rappuoli R, Grubeck-Loebenstein B. Ageing and immunity: addressing immune senescence to ensure healthy ageing. Vaccine 2010; **28**:3627–31.
2 Haynes L, Eaton SM, Burns EM, Randall TD, Swain SL. CD4 T cell memory derived from young naive cells functions well into old age, but memory generated from aged naive cells functions poorly. Proc Natl Acad Sci USA 2003; **100**: 15053–8.
3 Pawelec G, Barnett Y, Forsey R *et al.* T cells and aging, January 2002 update. Front Biosci 2002; **7**:d1056–183.
4 Pawelec G, Derhovanessian E. Role of CMV in immune senescence. Virus Res 2011; **157**:175–9.
5 Khurana S, Frasca D, Blomberg B, Golding H. AID activity in B cells strongly correlates with polyclonal antibody affinity maturation in-vivo following pandemic 2009-H1N1 vaccination in humans. PLOS Pathog 2012; **8**:e1002920.
6 van Dijk-Hard I, Soderstrom I, Feld S, Holmberg D, Lundkvist I. Age-related impaired affinity maturation and differential D-JH gene usage in human VH6-expressing B lymphocytes from healthy individuals. Eur J Immunol 1997; **27**:1381–6.
7 Frasca D, Landin AM, Lechner SC *et al.* Aging down-regulates the transcription factor E2A, activation-induced cytidine deaminase, and Ig class switch in human B cells. J Immunol 2008; **180**:5283–90.
8 Wang X, Stollar BD. Immunoglobulin VH gene expression in human aging. Clin Immunol 1999; **93**:132–42.
9 Frasca D, Diaz A, Romero M, Landin AM, Blomberg BB. High TNF-alpha levels in resting B cells negatively correlate with their response. Exp Gerontol 2014; **54**:116–22.
10 Frasca D, Diaz A, Romero M, Landin AM, Blomberg BB. Cytomegalovirus (CMV) seropositivity decreases B cell responses to the influenza vaccine. Vaccine 2015; **33**:1433–9.
11 Frasca D, Diaz A, Romero M *et al.* Intrinsic defects in B cell response to seasonal influenza vaccination in elderly humans. Vaccine 2010; **28**:8077–84.
12 Frasca D, Diaz A, Romero M, Mendez NV, Landin AM, Blomberg BB. Effects of age on H1N1-specific serum IgG1 and IgG3 levels evaluated during the 2011-2012 influenza vaccine season. Immun Ageing 2013; **10**:14.
13 Frasca D, Diaz A, Romero M *et al.* Unique biomarkers for B-cell function predict the serum response to pandemic H1N1 influenza vaccine. Int Immunol 2012; **24**:175–82.
14 Sayegh CE, Quong MW, Agata Y, Murre C. E-proteins directly regulate expression of activation-induced deaminase in mature B cells. Nat Immunol 2003; **4**:586–93.
15 Gibson KL, Wu YC, Barnett Y *et al.* B-cell diversity decreases in old age and is correlated with poor health status. Aging Cell 2009; **8**:18–25.
16 Wang C, Liu Y, Xu LT *et al.* Effects of aging, cytomegalovirus infection, and EBV infection on human B cell repertoires. J Immunol 2014; **192**:603–11.
17 Jiang N, He J, Weinstein JA *et al.* Lineage structure of the human antibody repertoire in response to influenza vaccination. Sci Transl Med 2013; **5**:171ra19.

18 Dunn-Walters DK, Ademokun AA. B cell repertoire and ageing. Curr Opin Immunol 2010; **22**:514–20.
19 Frasca D, Diaz A, Romero M, Ferracci F, Blomberg BB. MicroRNAs miR-155 and miR-16 decrease AID and E47 in B cells from elderly individuals. J Immunol 2015; **195**:2134–40.
20 Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci 2014; **69**:S4–9.
21 Frasca D, Diaz A, Romero M, Landin AM, Blomberg BB. Cytomegalovirus (CMV) seropositivity decreases B cell responses to the influenza vaccine. Vaccine 2015; **33**:1433–9.
22 Derhovanessian E, Theeten H, Hahnel K, Van Damme P, Cools N, Pawelec G. Cytomegalovirus-associated accumulation of late-differentiated CD4 T-cells correlates with poor humoral response to influenza vaccination. Vaccine 2013; **31**:685–90.
23 Trzonkowski P, Mysliwska J, Szmit E *et al.* Association between cytomegalovirus infection, enhanced proinflammatory response and low level of anti-hemagglutinins during the anti-influenza vaccination – an impact of immunosenescence. Vaccine 2003; **21**:3826–36.
24 Derhovanessian E, Larbi A, Pawelec G. Biomarkers of human immunosenescence: impact of Cytomegalovirus infection. Curr Opin Immunol 2009; **21**:440–5.
25 McElhaney JE, Zhou X, Talbot HK *et al.* The unmet need in the elderly: how immunosenescence, CMV infection, co-morbidities and frailty are a challenge for the development of more effective influenza vaccines. Vaccine 2012; **30**:2060–7.
26 Ahima RS. Connecting obesity, aging and diabetes. Nat Med 2009; **15**:996–7.
27 Minamino T, Orimo M, Shimizu I *et al.* A crucial role for adipose tissue p53 in the regulation of insulin resistance. Nat Med 2009; **15**:1082–7.
28 Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006; **444**:860–7.
29 Johnson AM, Olefsky JM. The origins and drivers of insulin resistance. Cell 2013; **152**:673–84.
30 Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest 2006; **116**:1793–801.
31 Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 2008; **371**:569–78.
32 Setty AR, Curhan G, Choi HK. Obesity, waist circumference, weight change, and the risk of psoriasis in women: Nurses' Health Study II. Arch Intern Med 2007; **167**:1670–5.
33 Casas R, Sacanella E, Estruch R. The immune protective effect of the Mediterranean diet against chronic low-grade inflammatory diseases. Endocr Metab Immune Disord Drug Targets 2014; **14**:245–54.
34 Hass DJ, Brensinger CM, Lewis JD, Lichtenstein GR. The impact of increased body mass index on the clinical course of Crohn's disease. Clin Gastroenterol Hepatol 2006; **4**:482–8.
35 Karlsson EA, Beck MA. The burden of obesity on infectious disease. Exp Biol Med 2010; **235**:1412–24.
36 O'Shea D, Corrigan M, Dunne MR *et al.* Changes in human dendritic cell number and function in severe obesity may contribute to increased susceptibility to viral infection. Int J Obes 2013; **37**:1510–3.
37 Anaya DA, Dellinger EP. The obese surgical patient: a susceptible host for infection. Surg Infect 2006; **7**:473–80.
38 Choban PS, Flancbaum L. The impact of obesity on surgical outcomes: a review. J Am Coll Surg 1997; **185**:593–603.
39 Nishimura S, Manabe I, Nagasaki M *et al.* CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med 2009; **15**:914–20.
40 Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 2007; **117**:175–84.
41 McLaughlin T, Deng A, Yee G *et al.* Inflammation in subcutaneous adipose tissue: relationship to adipose cell size. Diabetologia 2010; **53**:369–77.
42 Winer DA, Winer S, Shen L *et al.* B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. Nat Med 2011; **17**:610–7.
43 Duffaut C, Galitzky J, Lafontan M, Bouloumie A. Unexpected trafficking of immune cells within the adipose tissue during the onset of obesity. Biochem Biophys Res Commun 2009; **384**:482–5.
44 Nikolajczyk BS. B cells as under-appreciated mediators of non-auto-immune inflammatory disease. Cytokine 2010; **50**:234–42.
45 Nikolajczyk BS, Jagannathan-Bogdan M, Shin H, Gyurko R. State of the union between metabolism and the immune system in type 2 diabetes. Genes Immun 2011; **12**:239–50.
46 Shulzhenko N, Morgun A, Hsiao W *et al.* Crosstalk between B lymphocytes, microbiota and the intestinal epithelium governs immunity versus metabolism in the gut. Nat Med 2011; **17**:1585–93.
47 Winer DA, Winer S, Chng MH, Shen L, Engleman EG. B Lymphocytes in obesity-related adipose tissue inflammation and insulin resistance. Cell Mol Life Sci 2014; **71**:1033–43.
48 DeFuria J, Belkina AC, Jagannathan-Bogdan M *et al.* B cells promote inflammation in obesity and type 2 diabetes through regulation of T-cell function and an inflammatory cytokine profile. Proc Natl Acad Sci USA 2013; **110**:5133–8.
49 Frasca D, Ferracci F, Diaz A, Romero M, Lechner S, Blomberg BB. Obesity decreases B cell responses in young and elderly individuals. Obesity (Silver Spring) 2016; **24**:615–25.
50 Basu R, Breda E, Oberg AL *et al.* Mechanisms of the age-associated deterioration in glucose tolerance: contribution of alterations in insulin secretion, action, and clearance. Diabetes 2003; **52**:1738–48.
51 Folsom AR, Kaye SA, Sellers TA *et al.* Body fat distribution and 5-year risk of death in older women. JAMA 1993; **269**:483–7.
52 Huffman DM, Barzilai N. Role of visceral adipose tissue in aging. Biochim Biophys Acta 2009; **1790**:1117–23.
53 Einstein FH, Fishman S, Bauman J *et al.* Enhanced activation of a 'nutrient-sensing' pathway with age contributes to insulin resistance. FASEB J 2008; **22**:3450–7.
54 Gabriely I, Ma XH, Yang XM, Rossetti L, Barzilai N. Leptin resistance during aging is independent of fat mass. Diabetes 2002; **51**:1016–21.
55 Ruhl CE, Everhart JE, Ding J *et al.* Serum leptin concentrations and body adipose measures in older black and white adults. Am J Clin Nutr 2004; **80**:576–83.
56 Van Den Saffele JK, Goemaere S, De Bacquer D, Kaufman JM. Serum leptin levels in healthy ageing men: are decreased serum testosterone and increased adiposity in elderly men the consequence of leptin deficiency? Clin Endocrinol 1999; **51**:81–8.
57 Hukshorn CJ, Saris WH. Leptin and energy expenditure. Curr Opin Clin Nutr Metab Care 2004; **7**:629–33.
58 La Cava A, Matarese G. The weight of leptin in immunity. Nat Rev Immunol 2004; **4**:371–9.

59 Loffreda S, Yang SQ, Lin HZ *et al.* Leptin regulates proinflammatory immune responses. FASEB J 1998; **12**:57–65.
60 Zarkesh-Esfahani H, Pockley AG, Wu Z, Hellewell PG, Weetman AP, Ross RJ. Leptin indirectly activates human neutrophils via induction of TNF-alpha. J Immunol 2004; **172**:1809–14.
61 Agrawal S, Gollapudi S, Su H, Gupta S. Leptin activates human B cells to secrete TNF-alpha, IL-6, and IL-10 via JAK2/STAT3 and p38MAPK/ERK1/2 signaling pathway. J Clin Immunol 2011; **31**:472–8.
62 Lam QL, Wang S, Ko OK, Kincade PW, Lu L. Leptin signaling maintains B-cell homeostasis via induction of Bcl-2 and Cyclin D1. Proc Natl Acad Sci USA 2010; **107**:13812–7.
63 Procaccini C, De Rosa V, Galgani M *et al.* Leptin-induced mTOR activation defines a specific molecular and transcriptional signature controlling CD4+ effector T cell responses. J Immunol 2012; **189**:2941–53.
64 Procaccini C, De Rosa V, Galgani M *et al.* An oscillatory switch in mTOR kinase activity sets regulatory T cell responsiveness. Immunity 2010; **33**:929–41.
65 Lin Y, Damjanovic A, Metter EJ *et al.* Age-associated telomere attrition of lymphocytes in vivo is co-ordinated with changes in telomerase activity, composition of lymphocyte subsets and health conditions. Clin Sci 2015; **128**:367–77.
66 Son NH, Murray S, Yanovski J, Hodes RJ, Weng N. Lineage-specific telomere shortening and unaltered capacity for telomerase expression in human T and B lymphocytes with age. J Immunol 2000; **165**:1191–6.
67 Najarro K, Nguyen H, Chen G *et al.* Telomere length as an indicator of the robustness of B- and T-cell response to influenza in older adults. J Infect Dis 2015; **212**:1261–9.
68 Tzanetakou IP, Katsilambros NL, Benetos A, Mikhailidis DP, Perrea DN. 'Is obesity linked to aging?': adipose tissue and the role of telomeres. Ageing Res Rev 2012; **11**:220–9.
69 Al Snih S, Ottenbacher KJ, Markides KS, Kuo YF, Eschbach K, Goodwin JS. The effect of obesity on disability vs mortality in older Americans. Arch Intern Med 2007; **167**:774–80.
70 McAuley P, Myers J, Abella J, Froelicher V. Body mass, fitness and survival in veteran patients: another obesity paradox? Am J Med 2007; **120**:518–24.
71 Arterburn DE, Crane PK, Sullivan SD. The coming epidemic of obesity in elderly Americans. J Am Geriatr Soc 2004; **52**:1907–12.
72 Masoro EJ. Overview of caloric restriction and ageing. Mech Ageing Dev 2005; **126**:913–22.
73 Ahmet I, Wan R, Mattson MP, Lakatta EG, Talan M. Cardioprotection by intermittent fasting in rats. Circulation 2005; **112**: 3115–21.
74 Hursting SD, Lavigne JA, Berrigan D, Perkins SN, Barrett JC. Calorie restriction, aging, and cancer prevention: mechanisms of action and applicability to humans. Ann Rev Med 2003; **54**: 131–52.
75 Stern JS, Gades MD, Wheeldon CM, Borchers AT. Calorie restriction in obesity: prevention of kidney disease in rodents. J Nutr 2001; **131**:913S–7S.
76 Heilbronn LK, de Jonge L, Frisard MI *et al.* Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial. JAMA 2006; **295**:1539–48.
77 McGill AT, Stewart JM, Lithander FE, Strik CM, Poppitt SD. Relationships of low serum vitamin D3 with anthropometry and markers of the metabolic syndrome and diabetes in overweight and obesity. Nutr J 2008; **7**:4.
78 Karkeni E, Marcotorchino J, Tourniaire F *et al.* Vitamin D limits chemokine expression in adipocytes and macrophage migration *in vitro* and in male mice. Endocrinology 2015; **156**:1782–93.
79 Cherniack EP, Florez HJ, Troen BR. Emerging therapies to treat frailty syndrome in the elderly. Altern Med Rev 2007; **12**: 246–58.
80 Longo VD, Antebi A, Bartke A *et al.* Interventions to slow aging in humans: are we ready? Aging Cell 2015; **14**:497–510.
81 Mannick JB, Del Giudice G, Lattanzi M *et al.* mTOR inhibition improves immune function in the elderly. Sci Transl Med 2014; **6**:268ra179.