



## Review

## B cells and immunosenescence: A focus on IgG<sup>+</sup>IgD<sup>-</sup>CD27<sup>-</sup> (DN) B cells in aged humans

Matteo Bulati<sup>a</sup>, Silvio Buffa<sup>a</sup>, Giuseppina Candore<sup>a</sup>, Calogero Caruso<sup>a</sup>, Deborah K. Dunn-Walters<sup>b</sup>, Mariavaleria Pellicanò<sup>a</sup>, Yu-Chang Wu<sup>b</sup>, Giuseppina Colonna Romano<sup>a,\*</sup>

<sup>a</sup> Immunosenescence Unit, Department of Pathobiology and Medical and Forensic Biotechnologies, University of Palermo, Corso Tukory 211, 90134, Palermo, Italy

<sup>b</sup> Department of Immunobiology, King's College London Medical School, London, UK

## ARTICLE INFO

## Article history:

Received 27 August 2010

Received in revised form

13 December 2010

Accepted 15 December 2010

Available online 23 December 2010

## Keywords:

Immunosenescence

Memory B lymphocytes

Centenarian offspring

Alzheimer's disease

## ABSTRACT

Immunosenescence contributes to the decreased ability of the elderly to control infectious diseases, which is also reflected in their generally poor response to new antigens and vaccination. It is known that the T cell branch of the immune system is impaired in the elderly mainly due to expansion of memory/effector cells that renders the immune system less able to respond to new antigens. B lymphocytes are also impaired in the elderly in terms of their response to new antigens. In this paper we review recent work on B cell immunosenescence focusing our attention on memory B cells and a subset of memory B cells (namely IgG<sup>+</sup>IgD<sup>-</sup>CD27<sup>-</sup>) that we have demonstrated is increased in healthy elderly.

© 2010 Elsevier B.V. All rights reserved.

### 1. Introduction

In human ageing the ability to respond to vaccines and new infectious agents is impaired, mainly because of changes in adaptive immunity mediated by T and B cells. This phenomenon called 'immunosenescence' is influenced by environmental and genetic factors, but also by the antigenic load to which individuals are exposed throughout life, and this has an impact on immune performance in late life (Pawelec and Larbi, 2008; van Baarle et al., 2005).

Immunosenescence materially contributes to the decreased ability of the elderly to respond to new antigens and vaccinations and to control infectious diseases (Gardner et al., 2006; Genton et al., 2006). Indeed, ageing is associated with an increased incidence of infections such as viral influenza, respiratory syncytial virus (RSV) and pneumococcal pneumonia (Nicholson et al., 1997). Elderly people also have an increased incidence of bacterial infections in lungs, urinary tract, skin and other tissues and a higher incidence of tuberculosis and herpes zoster reactivation (Ginaldi et al., 2001). Vaccinations are powerful tools in order to prevent morbidity and mortality from infections in people over the age of 65 years, however, because of the impairment of immune functions with ageing, the currently available vaccines protect only

a small proportion of the elderly population (Weinberger et al., 2008).

Although most of the literature on immunosenescence have focused on T cell impairment, B cell compartment is also defective in the elderly: indeed humoral immune response is modified in the elderly both in the quality and quantity of the antibodies produced, and the number of circulating B cells is reduced in the aged (Cancro et al., 2009; Frasca et al., 2010b).

In addition it is known that, B cells have effector and regulatory functions other than antibody production (Sanz et al., 2007; Martin and Chan, 2006; Harris et al., 2000) and memory and naïve B cells can produce different cytokines and chemokines; in particular memory B cells produce high levels of the proinflammatory cytokines IL-1 $\alpha$ , IL-1 $\beta$ , IL-6 and TNF- $\alpha$  so suggesting that B cells might take part in the generation or in the maintenance of the inflammatory environment of the elderly (Agrawal and Gupta, 2010). Indeed a typical feature of ageing is the pro-inflammatory status observed in the elderly, related to chronic inflammatory diseases. So, ageing increases risk of disability and chronic diseases, with many older adults experiencing multiple chronic conditions in old age. Some evidence exists to support theories that increased health risks in old age are the result of environmental stressors that accumulate over time. These stressors potentially can disrupt the regulation of biological systems, however, not all individuals or population groups seem to be equally susceptible to the effects of stress. Questions remain as which factors mediate biological

\* Corresponding author. Tel.: +39 0916555906; fax: +39 0916555933.

E-mail addresses: [gcolonna@unipa.it](mailto:gcolonna@unipa.it), [taicol@katamail.com](mailto:taicol@katamail.com) (G. Colonna Romano).

responses to stress or genetic predisposition, lifestyle, or other factors. For example, it is believed that in order to achieve an advanced age, centenarians should be equipped with well preserved and efficient defense mechanisms, optimal combination of an appropriate genetic background and lifestyle (Franceschi et al., 1995). In these subjects, a higher frequency of genetic markers (polymorphisms) associated with a reduced pro-inflammatory ability seems to work against the onset of the main age-related disorders (cancer, dementia, diabetes and cardiovascular diseases) (Franceschi et al., 2007). On this basis, it might be an advantage to have centenarian parents; indeed it has been demonstrated that offspring of centenarians, who are in their 70s and 80s, have a survival advantage when compared to age-matched controls whose parents died at an average life expectancy. Centenarian offspring, like their parents, have genetic and functional advantages associated with lower cardiovascular disease risk (Terry et al., 2004a, 2004b). These findings support the hypothesis that centenarian offspring are inclined to healthy ageing and longer survival, making them a suitable target of ageing studies. Accordingly, a recent paper (Derhovanessian et al., 2010) has demonstrated that the typical hallmarks of immunosenescence are not present in subjects with familiar longevity.

Some diseases such as tumors, autoimmune phenomena, atherosclerosis, heart disease and Alzheimer's disease, are frequent in the late phase of the life and involve the dysregulation of immune parameters and/or chronic inflammation in their pathology (Candore et al., 2008; Vasto and Caruso, 2004). In fact, in frail elderly (i.e. Alzheimer's disease, AD, patients), two recent meta-analyses by our group have highlighted the role of cytokine polymorphisms in AD susceptibility, indicating the role of immune-inflammatory responses in AD (Di Bona et al., 2009, 2008). Furthermore, much evidences suggest the involvement of a systemic immune response in the pathogenesis of AD (Britschgi and Wyss-Coray, 2007; Speciale et al., 2007). Indeed, blood derived cells seem to accumulate in the AD brain (Rogers et al., 1998), while other studies have shown changes in the distribution and reactivity of immune cells in the blood (Pellicanò et al., 2010; Britschgi and Wyss-Coray, 2007; Monsonigo et al., 2003; Weksler et al., 2002).

On these basis in this paper we discuss the literature data on B cell immunosenescence and B cell memory focusing our attention on a subset of memory B cells (namely IgG<sup>+</sup>IgD<sup>-</sup>CD27<sup>-</sup>) that we have demonstrated is increased in healthy elderly (Colonna Romano et al., 2009), but not in centenarian offsprings (Colonna Romano et al., 2010) and Alzheimer's disease patients (*preliminary observations, see below*).

## 2. Immune system and ageing

### 2.1. Inflamm-aging and T lymphocytes

A typical feature of ageing is a chronic, low-grade inflammation characterized by a general increase in the production of pro-inflammatory cytokines and inflammatory markers (Cevenini et al., 2010). Indeed, elevated plasma concentrations of IL-6, IL-1 $\beta$ , and TNF- $\alpha$  have been described in elderly populations and were postulated as predictive markers of functional disability, frailty and mortality (Bruunsgaard et al., 2003; Ershler and Keller, 2000; O'Mahony et al., 1998) and it has been suggested that chronic inflammation supports the development and progression of age-related diseases, such as osteoporosis, neurodegeneration and atherosclerosis (Gao and Hong, 2008; Ginaldi et al., 2005; Libby, 2002). Subclinical inflammation may be caused by chronic stimulation of the innate immune system by degradation products and/or by the partial inability of the aged immune system to eliminate certain pathogens (Weinberger et al., 2009), this inflammatory sta-

tus may slowly damage one or several organs, especially when unfavorable genetic polymorphisms and epigenetic alterations are concomitant, leading to an increased risk of frailty together with the onset of age-related chronic diseases (reviewed by Cevenini et al., 2010 and Vasto et al., 2007). The age-dependent up-regulation of the inflammatory response has been termed "inflamm-aging" (Franceschi et al., 2000b, 2000c), due to both the chronic antigenic stimulation and the genetic background that render elderly prone to frailty (Balistreri et al., 2008, 2007; Franceschi et al., 2005; Lio et al., 2004; Pes et al., 2004).

T cell immunosenescence has been extensively studied and many details have been clarified (see reviews by McElhaney and Effros, 2009 and Pawelec and Larbi, 2008): the percentage and the absolute numbers of circulating CD3<sup>+</sup> T lymphocytes and of CD4<sup>+</sup> and CD8<sup>+</sup> T cell subsets decreases (Pawelec et al., 2002; Cossarizza et al., 1996; Sansoni et al., 1993) and there is a gradual shift from naïve CD45RA<sup>+</sup> to more activated or memory CD45RO<sup>+</sup> cells (Pawelec and Larbi, 2008; Pawelec et al., 2002) and it is believed that the decrease in naïve T cell numbers with age is the result of thymic involution in combination with ongoing differentiation of naïve T cells into antigen-experienced memory or effector cells (Appay et al., 2010).

One of the most remarkable qualitative changes in the memory T cell population during ageing is the appearance of clonally expanded CD8<sup>+</sup>CD28<sup>-</sup> T cells. Analysis of T cell receptor clonotypes, CD28 expression, telomere length and proliferative capacity has suggested that these cytotoxic T cells have reached replicative senescence (Pawelec and Larbi, 2008; Globerson and Effros, 2000). The presence of high proportions of senescent CD8<sup>+</sup>CD28<sup>-</sup> T cells would impact homeostatic mechanisms regulating the amount of memory and naïve T-cell subsets and hence would reduce the T cells available for an effective antiviral response (Pawelec and Larbi, 2008; Globerson and Effros, 2000; Fagnoni et al., 1996). These features are caused by persistent life-long antigenic stress that leads to the marked shrinkage of T cell repertoire diversity with age (Pawelec and Larbi, 2008; Pawelec et al., 2002; Wack et al., 1998). Moreover, it has been recently observed that the evaluation of the number of CD8<sup>+</sup>CD28<sup>-</sup> T cells is not merely an "immunological curio" as it correlates with frailty (Semba et al., 2005) and with an impaired response to influenza vaccination in the elderly (Weng et al., 2009). It has been proposed that CMV infection leads to the described changes of CD8 T cells in the elderly, causing both the shrinkage of the TCR repertoire and the accumulation of CD8<sup>+</sup>CD28<sup>-</sup> effector T cells. These cells can further stimulate the inflammatory processes by IFN $\gamma$  production as suggested (Almanzar et al., 2004) although others (Ouyang et al., 2003) have demonstrated that these cells are dysfunctional. The reduction of the costimulatory molecule CD28 has also been reported on CD4 T cells that show a defect in CD154 (CD40L) expression too, this in turn causes the reduced ability of CD4 T cells to provide help to B cells for both proliferation and Ig production.

Other authors have described a significant decrease of CD8<sup>+</sup> T cells with no significant changes in CD4<sup>+</sup> T cells with ageing, leading to an unexpected increase in the CD4:CD8 ratio (Gruber et al., 2007; Yan et al., 2010).

### 2.2. B lymphocytes

Although T cell alterations play a significant role in age-related immune changes, alterations in B cells also occur both in human and mice, indeed advanced age is accompanied by substantial changes in all B cell compartments and, consequently, humoral immune function. These changes include shifts in the magnitude of all B cell compartments, specificity repertoire changes, modified peripheral B cell dynamics, and weakened humoral responses (Miller and Cancro, 2007).

Before leaving the bone marrow, B cells pass through several stages of development and, any change in the different steps will result in a change in overall repertoire. In mice there is a decline in bone marrow output and impairments in haematopoietic stem cell (HSC) commitment to the B lineage (Guerrettaz et al., 2008; Miller and Allman, 2003), which is manifested as a deficit in the numbers of early B cell populations such as the pro, pre, transitional and mature naïve B cells. Others have suggested that it is not the bone marrow output by itself that is crucially impaired but rather the ability of naïve B cells from older mice to colonise peripheral compartments that is reduced (Johnson et al., 2002). Even though the reduced influx of fresh naïve cells into the periphery, the total numbers of peripheral B cells in mice remain the same (Miller and Cancro, 2007). There are several mechanisms that can explain this anomaly. It has been demonstrated that splenic B cells in old mice have a reduced turnover as compared to splenic B cells from young mice, and so live longer (Kline et al., 1999), this increased longevity may signal to the newly made B cells that the niche is full and requires no more cells (Minges Wols et al., 2009).

In contrast to mouse model, in humans there is a decrease both in percentage and absolute number of total CD19<sup>+</sup> B lymphocytes (Veneri et al., 2009; Faria et al., 2008; Frasca et al., 2008; Shi et al., 2005; Chong et al., 2005; Colonna Romano et al., 2003, 2002; Breitbart et al., 2002; Huppert et al., 1998; Wikby et al., 1994; Paganelli et al., 1992). Moreover, it has been demonstrated that absolute number of B cell precursors in the bone marrow declines with age and particularly during adolescence (McKenna et al., 2001), however, B lymphopoiesis persists throughout adult life (Rossi et al., 2003). In particular, in mice ratios between precursors and immature B cells within the bone marrow and mitotic activity were found to be remarkably constant during ageing (Riley et al., 1991).

As mentioned above, in elderly people there is a shrinkage of the T cell repertoire due to the chronic stimulation of the immune system by antigens and persistent infection by herpetic viruses such as CMV (Pawelec and Larbi, 2008; Vasto et al., 2007). On the B cell side, changes in the B cell repertoire have also been described and in old mice it has been reported a different usage of both V<sub>H</sub> and V<sub>L</sub> gene families that those used by young animals (Nicoletti et al., 1991). Old mice have also been shown to have altered repertoires in response to challenge with the hapten phosphorylcholine (Nicoletti et al., 1993, 1991) and, as in humans (see below), the response in young mice was oligoclonal, dominated by use of one particular immunoglobulin heavy chain variable region (IGHV) gene, whereas older mice had a more diverse response. Moreover, experiments using young and old donors to reconstitute immune-deficient SCID mice, suggest that these differences arise from B cell intrinsic factors, although the cytokine environment might also play a role (Shriner et al., 2006). In murine models it has also been reported the impaired expression of the surrogate  $\lambda$  chain and the increased usage of the VhS107 family that can also have consequences in the formation of the B cell repertoire (Alter-Wolf et al., 2009a). Some reports on B cell repertoire indicated that older mice showed evidence of non-malignant clonal expansion (LeMaoult et al., 1997), which in view of the homeostatic mechanisms that maintain the total numbers of B lymphocytes would lead to a decrease in diversity.

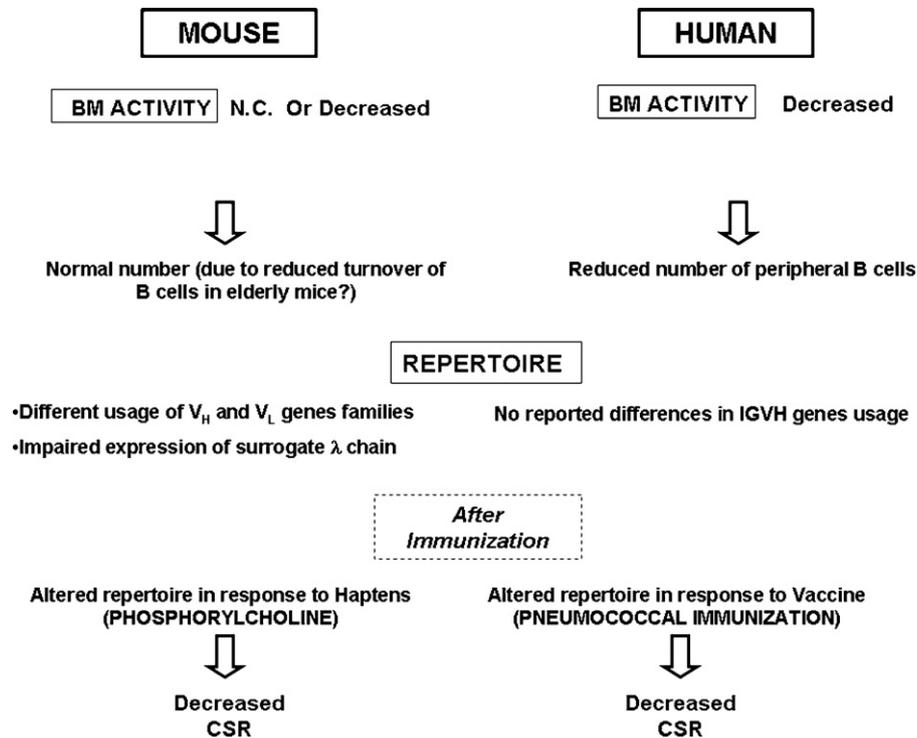
In humans after pneumococcal immunization the analysis of the V<sub>H</sub> chain repertoire has shown differences between young and elderly donors, a loss of oligoclonality and a reduced frequency of somatic mutation in the elderly (Kolibab et al., 2005). The same group also analysed the V<sub>L</sub> chain repertoire in response to the same immunization and has demonstrated significant differences in V<sub>L</sub> gene usage between young and old subjects: in fact the elderly preferentially use Vk4 in response to pneumococcal polysaccharide 4 and 14, while young donors use predominantly V $\lambda$ 3 and/or V $\lambda$ 1

genes, moreover oligoclonality of light chains was not so regular in the elderly as it has been demonstrated in the heavy gene usage and no detectable changes in the frequency of mutations have been reported for the L genes studied (Smithson et al., 2005). However the authors observe that these might be recall responses as adults have significant levels of antibodies against pneumococcal antigens before the treatment with the vaccine (Kolibab et al., 2005; Smithson et al., 2005).

Previous studies, concentrating on IGHV gene use, have not always shown significant age-related differences in the pre-immune B cell repertoire (Kolar et al., 2006; Wang and Stollar, 1999), indeed Banerjee et al. (2002), have shown that the somatic hypermutation process occurs at the same rate in young and old humans, and so the increased levels of mutations in Ig genes found in older people by others (Chong et al., 2003) is more likely a consequence of accumulation rather than altered rate. The consequence might be the collapse in B cell diversity in some elderly individuals that, as it has been recently demonstrated using the CDR3 spectratyping, is correlated with poor health status in the elderly (Gibson et al., 2009). A schematic illustration is shown in Fig. 1.

On the whole, it seems that the antibodies generated in old humans are less protective compared to the antibodies generated in young ones, as shown by their reduced ability to opsonize in vitro after vaccination with bacteria-derived polysaccharides (Schenkein et al., 2008). Moreover the anti-influenza response is reduced in the elderly after vaccination (Weinberger et al., 2008; Murasko et al., 2002) and there are also diminished recirculating long-lived antibody-secreting plasma cells in the bone marrow (Zheng et al., 1997; Manz et al., 1997). This might be related to a poor persistence of effective antibodies after vaccination in the elderly although care needs to be taken in interpretation here since the current data is limited and is from different vaccine formulations such as live-attenuated vaccines (e.g. against polio and measles) or killed microorganisms or toxoids (e.g. against tetanus) which may affect data on persistence.

In mice, some reports suggest that, with ageing, the levels of total and specific serum IgG and IgM increase (Koga et al., 2000), while other authors found a reduction in specific IgG and IgM in aged animals (Faria et al., 1998). Speziali et al. (2009), have recently shown that basal levels of total serum immunoglobulins, IgG and IgA were increased while antigen-specific immunoglobulins, IgG and IgA were reduced in aged mice compared to young ones. This divergence between total and specific levels of Immunoglobulins, IgG and IgA may reflect the biased repertoire of B cells in aged mice (Koga et al., 2000) and their inability to mount specific immune responses to new antigens. Moreover it has been shown that ageing is followed by an increased production of auto-antibodies in parallel with a diminished production of antibodies against foreign antigens (Weksler and Szabo, 2000). The skewed repertoire of B and T cells in aged animals probably result from the expansion of a limited number of memory cells that compensate for the reduction in the output of naïve B and T cells from bone marrow and thymus. The increased levels of non-specific immunoglobulins in aged mice seems to result from activation of memory cells already selected by previous encounters with antigens (Speziali et al., 2009). On other hand, both basal levels and antigen-specific IgM were not altered in old mice (Speziali et al., 2009) suggesting that class-switch-independent IgM responses, being low affinity reactions, are less affected by repertoire limitations. Concerning the effects of ageing on the number of immunoglobulin-producing cells in the bone marrow, Speziali et al. (2009) have shown that IgG, IgM and IgA immunoglobulin-producing cells were increased in aged mice and these results are coherent with the levels of serum immunoglobulins found, except for IgM that were unaltered in old mice. Frasca et al. (2004, 2007), have shown that in vitro stimulated splenic B cells from senescent mice are deficient in



**Fig. 1.** Bone marrow activity and B cell repertoire in elderly mice and humans compared with young individuals. N.C.: no change; and CSR: class switch recombination.

the production of multiple class switch isotypes and that deficiency is correlated with decreased induction of transcription factor E47, poor activation-induced cytidine deaminase (AID) expression and consequent down-regulation of class switch recombination. As others (Speziali et al., 2009) have reported increased levels of post-switch immunoglobulin isotypes as well as a rise in the number of IgA- and IgG-producing cells in aged mice, it could be speculate that this augment may be due to the proliferation of memory B cells, rather than activation of naïve cells. Accordingly Frasca et al. (2004), have demonstrated that B cell proliferation seems not to be affected by ageing. Increase in proliferation of already activated B cells may also explain why total but not antigen-specific IgG and IgA levels were found higher in older animals.

Also in aged humans a paradox exists in B cell biology; although there is a reduced number of peripheral B cells, and a reduced ability to produce antibodies “in vitro” (Frasca et al., 2008), the amount of “switched” Ig in the serum of elderly and centenarians is increased (Listì et al., 2006; Paganelli et al., 1992). Indeed, there is an age-related increase of IgG and IgA serum levels, whereas IgM level decrease or remain unchanged and IgD decrease with age (Listì et al., 2006; Paganelli et al., 1992). However, in vitro stimulation of human peripheral blood B cells by anti-CD40 and IL-4 induces a lower production of IgG from elderly donors, correlated to a reduction of the transcription factor E47 and AID in old people (Frasca et al., 2008). Moreover the same authors have recently demonstrated that the level of AID in response to polyclonal stimulation by CpG can predict the size of the response to influenza vaccination (Frasca et al., 2010a).

There are still many contradictory observations on B cells and antibodies with age, as shown in Table 1.

### 3. Markers of memory B lymphocytes

Memory lymphocytes are crucial cells in the immune system: facilitating a recall (anamnestic) response to previously encountered antigens. The “memory topic” is an interesting field for

immunologists involved in ageing studies, as it has been suggested that the filling of the immunological space with memory T cells renders the immune system less able to respond to new antigens (Franceschi et al., 2000a). On the other side, the ability to produce memory cells is essential for effective vaccination (Weinberger et al., 2008).

Memory B cells can be discriminated from naïve ones by the presence of somatic hypermutations in the variable gene sequences of their Ig (Klein et al., 1998). Moreover, to easily discriminate between naïve and memory B cells, phenotypic markers as surface immunoglobulins (IgD, IgM, IgG, IgA) and CD27 are currently used. Previously, IgD<sup>+</sup> cells were classified as naïve cells and IgD<sup>-</sup> cells as memory B cells (Black et al., 1978), however, the description of an IgD<sup>+</sup> subset expressing somatic hypermutations of the Ig genes discontinued its use for the unequivocal identification of naïve and memory B cells (Klein et al., 1998).

Also CD27 was commonly used as a marker of human memory B cells, because its expression is correlated with the presence of somatic hypermutations in Ig genes (Agematsu et al., 2000), in spite of this, many authors have recently demonstrated the presence in the blood of memory B cells that lack CD27 (Colonna Romano et al., 2009; Frasca et al., 2008; Wei et al., 2007; Fecteau et al., 2006; Anolik et al., 2004). The disconnected expression of CD27 and IgG has been demonstrated by Fecteau et al. (2006), and we found the same results in our samples evaluating the expression of CD27 on gated IgG<sup>+</sup> magnetically sorted B cells, or the expression of IgG on gated CD27<sup>+</sup> or CD27<sup>-</sup> magnetically sorted B lymphocytes. Indeed, not all IgG<sup>+</sup> cells were CD27<sup>+</sup>, in addition the CD27<sup>-</sup> B population also contains a small proportion of IgG-switched B cells (*unpublished observations*).

So naïve B cells are identified as IgG<sup>-</sup> IgA<sup>-</sup> IgD<sup>+</sup> CD27<sup>-</sup>, whereas the memory B cell population seems to be very heterogeneous, comprising three types: “IgM memory” cells that are IgD<sup>+</sup> IgM<sup>+</sup> CD27<sup>+</sup> (Klein et al., 1998), also identified as IgD<sup>+</sup> CD27<sup>+</sup> “unswitched memory” by Shi et al. (2005), “classical” switched memory IgG<sup>+</sup>/IgA<sup>+</sup> CD27<sup>+</sup>, and the IgG<sup>+</sup>/IgA<sup>+</sup> CD27<sup>-</sup> B cells (Fecteau

**Table 1**  
Main modifications of B cells and B cells products in elderly human.

B cells or B cells products	Changes	References
Total B cells (percentage)	Decrease	Faria et al. (2008) (dramatic decrease in over 75 years old), Frasca et al. (2008), Colonna Romano et al. (2003), Wikby et al. (1994), Paganelli et al. (1992)
Total B cells (absolute number)	Decrease	Veneri et al. (2009), Frasca et al. (2008), Shi et al. (2005), Chong et al. (2005), Breitbart et al. (2002), Huppert et al. (1998), Wikby et al. (1994), Paganelli et al. (1992)
CD19 <sup>+</sup> CD5 <sup>+</sup> B1 cells (percentage and absolute number)	Increase Decrease	Weksler (2000) Colonna Romano et al. (2002)
Total Ig [after specific immunization]	No change [Decrease]	Listi et al. (2006), Chong et al. (2005), Globerson and Effros (2000), Weksler (2000), Weksler and Szabo (2000) [Weksler, 2000; Frasca et al., 2005]
IgG (/ml) [after specific immunization]	No change Increase [Decrease]	Chong et al. (2005) Frasca et al. (2008), Listi et al. (2006), Paganelli et al. (1992) [Frasca et al., 2005, 2008]
IgA	No change Increase	Chong et al. (2005) Listi et al. (2006), Paganelli et al. (1992)
IgM [after specific immunization]	Decrease No change [Decrease]	Listi et al. (2006), Chong et al. (2005) Paganelli et al. (1992)
IgE [after specific immunization]	No change [Decrease]	[Shi et al., 2005] Listi et al. (2006), Chong et al. (2005)
IgD	Decrease	[Frasca et al., 2005]
Autoantibodies	Increase	Listi et al. (2006) Globerson and Effros (2000), Weksler (2000), Weksler and Szabo (2000), Candore et al. (1997)

et al., 2006), called also double negative (DN) B cells due to the lack of both IgD and CD27 (Colonna Romano et al., 2009).

Following antigenic challenge naïve B cells can differentiate into low-affinity Ig-secreting cells or mature within a germinal center into high-affinity memory cells expressing different Ig isotypes (Wolniak et al., 2004). Classically T cell help is required for memory B cell generation, germinal center formation, isotype switching and somatic hypermutation, and helping is, mainly provided following the binding of CD154 (CD40L) expressed on T cells with CD40 expressed on B cells (Wolniak et al., 2004). The importance of CD40 stimulation in these events has been demonstrated in X-linked hyper IgM syndrome patients, in which CD40–CD154 interaction is defective (Lougaris et al., 2005). In these patients, germinal center formation and the memory B cell response are strictly impaired, although a peripheral B cell fraction expressing CD27 persists (Weller et al., 2001), supporting the hypothesis that this marker does not exclusively identify classical germinal center-derived memory B cells: this CD27<sup>+</sup> (also IgM<sup>+</sup>IgD<sup>+</sup>) compartment was recognized as the peripheral counterpart of splenic marginal zone (MZ) B cells (Weller et al., 2005). Although expressing mutated Ig genes, this subset differs from classical memory B cells, since hypermutation can occur in absence of CD40–CD154 interactions and the two populations have a different Ig gene repertoire (Weller et al., 2005; Wu et al., 2010). Indeed, unlike classical memory B cells, the IgM<sup>+</sup>IgD<sup>+</sup>CD27<sup>+</sup> subset is dedicated to immune responses against T-independent antigens (Weller et al., 2004; Krutzmann et al., 2003; Spencer et al., 1998) during which they act as innate effectors in the first line of defense but not as memory cells (Pillai et al., 2005, 2004; Weller et al., 2005, 2004).

On the other hand, the requirement for CD27 expression on classical memory B cells has never been firmly established in humans (Tangye and Hodgkin, 2004). Indeed, as reported by Fecteau and Néron (2003), prolonged CD40 stimulation triggered naïve B cells to switch to IgG and to express CD27 even in absence of somatic hypermutation, suggesting that these events could be independent. The CD27 molecule in mice, rather than a memory marker, appears to be expressed in recent B cell activation and is not absolutely necessary for secondary responses (Xiao et al., 2004). These results suggest that CD27 is not “the” marker of memory B cells and that

the identification of naïve and memory B cells is not strictly related to this marker.

It has been reported (Anolik et al., 2004), that patients with systemic lupus erythematosus (SLE) show an increase of DN B cells that is correlated with the disease activity index (Wei et al., 2007). The Authors report that these B cells, that are IgG or IgA positive, show a degree of hypermutation of V<sub>H</sub> gene comparable to CD27<sup>+</sup> unswitched memory cells. Moreover they suggest that these CD27<sup>−</sup> memory B cells could represent either progenitors, or the progeny, of CD27<sup>+</sup> memory cells that fail to go through a productive germinal center reaction. Thus the hypothesis that CD27<sup>−</sup> memory B cells might develop outside the germinal center, perhaps in extrafollicular reactions capable of supporting hypermutation as was demonstrated in mice (William et al., 2002). Interestingly, dendritic cells have been shown to activate extrafollicular B cells and are also known to induce isotype switching in a CD40-independent way through BLyS–BAFF–R interaction (Qi et al., 2006) so this could be also the case for CD27<sup>−</sup> memory B cells. In our previous paper (Colonna Romano et al., 2009), we report that DN cells show low expression of the CD40 molecule, so these might not cooperate with T cells. Indeed, the finding that IgG<sup>+</sup> memory B cells in mice can be generated following T cell-independent responses corroborates the likelihood that this mechanism is also active in humans (Obukhanych and Nussenzweig, 2006).

Alternatively, CD27<sup>−</sup> cells might represent activated follicular cells that initiate the germinal center reaction after receiving early CD154-mediated T cell help, but fail to progress through this pathway, so explaining their failure to acquire CD27 and their lower rate of somatic hypermutation as compared with CD27<sup>+</sup> classical memory B cells. Given that CD27 interacts with CD70 on activated T cells, it is believable that the absence of CD27 might impair the ability of these cells to receive the complete and persistent degree of T cell help required to complete a germinal center reaction (Toellner et al., 2002). Interestingly, somatic hypermutation in the absence of CD27 is present in two different B cell tumors as Waldenström's macroglobulinemia and hairy cell leukemia, for which an extra-germinal center derivation has been proposed (Kriangkum et al., 2004; Forconi et al., 2004).

Moreover, to investigate whether IgG<sup>+</sup>CD27<sup>−</sup> cells corresponded to post-germinal center cells or not, Fecteau et al. (2006)

evaluated their frequency of somatic hypermutation compared to IgG<sup>+</sup>CD27<sup>+</sup> classical memory B cells. IgG<sup>+</sup>CD27<sup>-</sup> had significantly lower mutation levels than did IgG<sup>+</sup>CD27<sup>+</sup> cells. The low frequency of somatic mutations in IgG<sup>+</sup>CD27<sup>-</sup> cells may support the theory that this population could emerge independently from T cell help or from CD40–CD154 interaction in humans (Weller et al., 2001).

#### 4. Naïve and double negative B lymphocytes in healthy aged, centenarian offsprings and Alzheimer's disease patients

Humoral memory is generally viewed as supported by two cellular compartments: the effector memory compartment, represented by antibody-producing plasma cells and the central memory one, represented by memory B cells that are precursors capable of generating and replenishing the plasma cell compartment. However, B cells have effector and regulatory functions other than antibody production such as T cell and dendritic cell regulation and cytokine and chemokine production (Sanz et al., 2007; Martin and Chan, 2006; Harris et al., 2000) indeed it has been demonstrated that memory B cells produce great amounts of proinflammatory cytokines and so might play a role in the generation or in the maintenance of the inflammatory environment of the elderly (Agrawal and Gupta, 2010). In consideration of this and also in the light of the inflammatory environment in successful and unsuccessful ageing, here we focus on literature data of the naïve/memory compartment in the elderly. In previous papers we (Colonna Romano et al., 2009, 2003) and others (Gupta et al., 2005) have demonstrated a reduction of naïve CD27<sup>-</sup> B cells in the elderly and no significant reciprocal increase in CD27<sup>+</sup> memory B cells (Klein et al., 1998; Agematsu et al., 2000). On the other hand, Shi et al. (2005) have shown that CD27<sup>+</sup> memory B cells, particularly the IgD<sup>+</sup>IgM<sup>+</sup>CD27<sup>+</sup> “IgM memory” B cells, decline dramatically in elderly subjects. These data are in agreement with reduced output of B cells. Besides, as it has been proposed that IgM memory B cells are involved in the response to pneumococcal infection, this reduction is considered responsible for the increased susceptibility to bacterial infections. Furthermore, other groups (Frasca et al., 2008; Shi et al., 2005) have demonstrated the increase of naïve B cells in the elderly. Frasca et al. (2008), described peripheral blood naïve B cells [CD19<sup>+</sup>CD27<sup>-</sup>IgG<sup>-</sup>IgA<sup>-</sup> (presumably IgM<sup>+</sup>)] by lack of switched receptor rather than by the presence of IgD; they report the increase of the percentage and no changes of the absolute number of these cells in the elderly. As we have reported (Colonna Romano et al., 2009) the decrease of naïve B cells in the elderly (identified as IgD<sup>+</sup>CD27<sup>-</sup>) and the increase of IgD<sup>-</sup>CD27<sup>-</sup>, and in order to better characterize the DN B cells (IgD<sup>-</sup>CD27<sup>-</sup>), we are studying the expression on these cells of IgG and IgM. Our preliminary results show that, in our samples, the percentage of IgG<sup>-</sup>IgM<sup>+</sup> B cells is a very small fraction (~5%) of total IgD<sup>-</sup>CD27<sup>-</sup> DN B cells. However, further analysis (e.g. the expression of IgA) and the specific study of these cells in the elderly are necessary to better understand how this very small fraction changes with ageing, and whether the change of these cells might influence the amount of “naïve” B cells identified as IgG<sup>-</sup>IgA<sup>-</sup>CD27<sup>-</sup>. In the same paper (Frasca et al., 2008) and in a following published review (Frasca et al., 2010b) they report the decrease of both percentage and absolute number of switched memory B cells (IgG<sup>+</sup>/IgA<sup>+</sup>/CD27<sup>+</sup> or CD27<sup>-</sup>). We never found this result evaluating B memory cells as IgD<sup>-</sup>CD27<sup>+</sup> or CD27<sup>-</sup> although, due the reduced number of total B cells in the elderly, the absolute number of memory B cells is reduced also in our old subjects.

Centenarian offspring (CO) can be considered people who might be genetically advantaged for successful ageing, and Alzheimer's disease patients as a model of unsuccessfully aged (Terry et al.,

**Table 2**

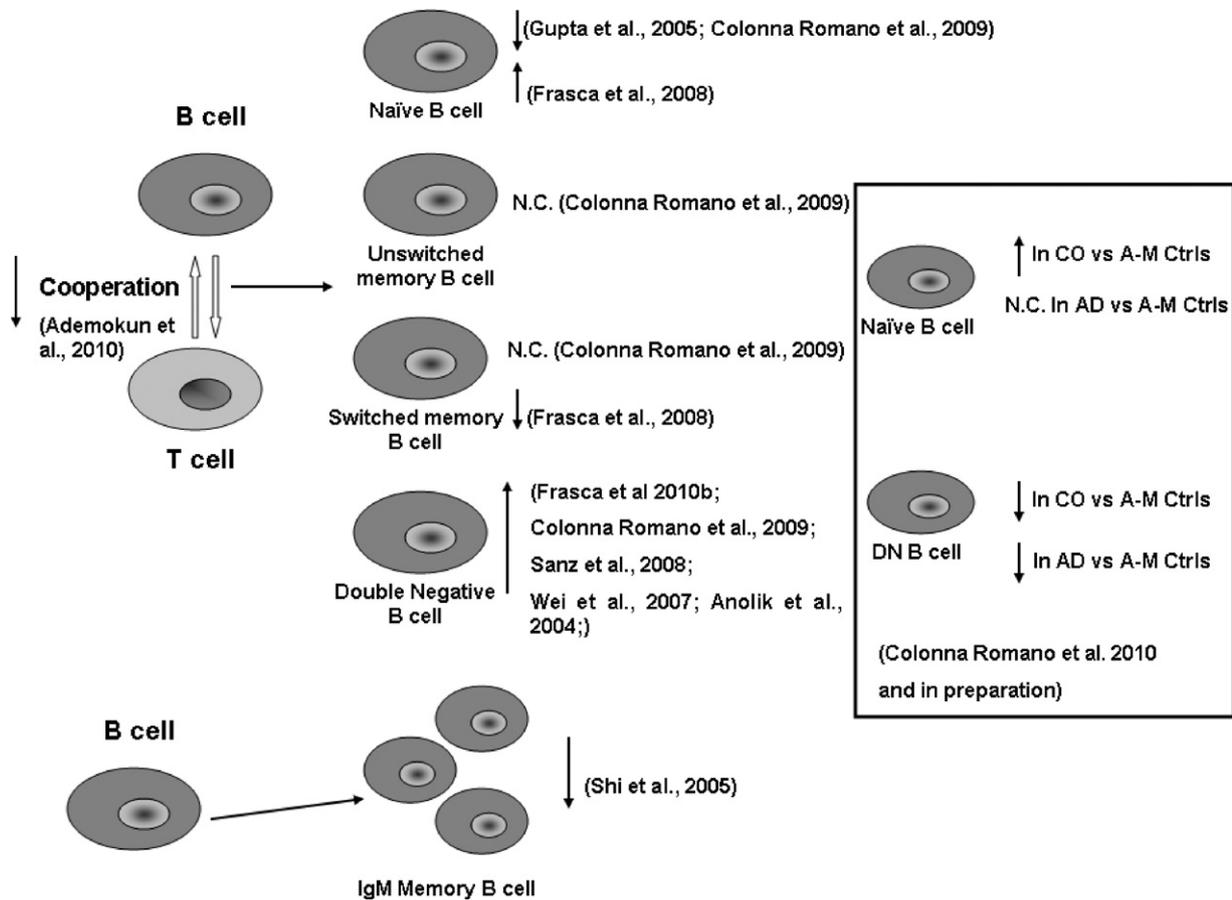
Analysis of IgA, IgG and IgM serum concentrations in centenarian offspring ( $n=29$ , age range 59–83) and age-matched controls ( $n=25$ , age range 60–85). The serum of all subjects was stored in aliquots at  $-80^{\circ}\text{C}$  until analysis and the immunoglobulin concentrations were determined by Integra 800 (Roche Diagnostics, Milan, Italy) as described (Colonna Romano et al., 2010). Data are expressed as mean  $\pm$  SD. Significance has been evaluated by ANOVA Test (values statistically significant when  $p < 0.05$ ).

Serum immunoglobulin concentrations (mg/dl)	Centenarian offspring ( $n=29$ ) [mean $\pm$ SD]	Age matched controls ( $n=25$ ) [mean $\pm$ SD]	$p$
IgA	250 $\pm$ 25	280 $\pm$ 25	0.2
IgG	1080 $\pm$ 120	950 $\pm$ 115	0.4
IgM	150 $\pm$ 20	80 $\pm$ 20	0.0004

2004a, 2004b; Derhovanessian et al., 2010; Di Bona et al., 2009, 2008).

We do not show any significant changes, neither in percentage nor in absolute number of B cells, between centenarian offspring and their “normal” age-matched controls (Colonna Romano et al., 2010). However, Alzheimer's disease patients show a significant reduction in B cells (both in percentage and absolute number) when compared with healthy elderly (Pellicanò et al., 2010; Speciale et al., 2007). As mentioned looking at different B cell subsets, we have shown a significant decrease of the percentage of IgD<sup>+</sup>CD27<sup>-</sup> naïve B cells in old compared to young donors ( $42.2 \pm 3.6$  in young,  $33.3 \pm 2.7$  in elderly,  $p < 0.05$ ; data are expressed as mean  $\pm$  SEM), no differences in unswitched (IgD<sup>+</sup>CD27<sup>+</sup>) and switched (IgD<sup>-</sup>CD27<sup>+</sup>) memory compartment and a significant increase of the percentage of IgD<sup>-</sup>CD27<sup>-</sup> DN B cells ( $6 \pm 0.6$  in young,  $15.8 \pm 1.4$  in elderly,  $p < 0.0001$ ) (Colonna Romano et al., 2009). Our data in centenarian subjects shows the same trend of elderly people of naïve ( $47.6 \pm 8.4$ ) and IgD<sup>-</sup>CD27<sup>-</sup> ( $11.1 \pm 4.7$ ) B lymphocytes. In order to gain insight in the biological relevance of B cell subset age-related changes, we have compared (Colonna Romano et al., 2010; Bulati et al., 2008) memory/naïve B cell populations in centenarian offsprings to those in old age-matched control. In the former, we do not observe the typical naïve-memory shift observed in the elderly and in centenarians. Indeed, as previous demonstrated, IgD<sup>+</sup>CD27<sup>-</sup> naïve B cells are significantly increased ( $50.3 \pm 3.3$ ,  $p = 0.05$ ) in CO when compared to age-matched controls, while the IgD<sup>-</sup>CD27<sup>-</sup> DN B cells are significantly reduced ( $7.4 \pm 0.9$ ,  $p = 0.003$ ). So, B cell subsets of CO are similar to those observed in young subjects previously described (Colonna Romano et al., 2009). This observation is strengthened by serum immunoglobulin measurement. In fact, the concentration of IgM, a marker of the primary response, shows significant higher levels in centenarian offspring when compared to age-matched controls, whereas IgG and IgA levels are not significantly different (Table 2) (Colonna Romano et al., 2010). Also in this case, the values are within the range of the level observed in young subjects (Listì et al., 2006) supporting the “youth” of B cell branch in these subjects.

Concerning the model of unsuccessful ageing, we are studying the distribution of B lymphocytes subsets in AD patients, comparing the results to data obtained in age-matched healthy controls. Our preliminary results show that no significant differences in the IgD<sup>+</sup>CD27<sup>-</sup> naïve B cells compartment between the two groups, as well as in unswitched (IgD<sup>+</sup>CD27<sup>+</sup>) and switched (IgD<sup>-</sup>CD27<sup>+</sup>) memory pool, are observed while there is a significant decrease of IgD<sup>-</sup>CD27<sup>-</sup> DN B cells in AD compared to age-matched controls ( $6.1 \pm 0.7$  vs.  $15.8 \pm 1.4$ ,  $p < 0.0001$ ). The finding that this population is not increased in AD subjects, as in age matched healthy controls, together with the observation that all others B cell subsets are unmodified, although both the percentage and the absolute number of B cells are decreased (Pellicanò et al., 2010), need further



**Fig. 2.** Naïve and memory B cell compartment in humans as reported by different authors N.C.: no change; CO: centenarian offspring; AD: Alzheimer's disease patients; and A-M Ctrls: age-matched controls.

investigation. Moreover, this is an interesting result, as Speciale et al. (2007) have shown a reduction of  $CD8^+CD28^-$  T cells in patients affected by AD. These cells, as mentioned, are increased in elderly and are considered to be exhausted late memory effector cells (Pawelec and Larbi, 2008). So, this might support the idea that DN B cells are also exhausted cells. The reported data are summarized in Fig. 2.

## 5. Conclusions

It has been shown that both T cell-mediated cellular and B cell-mediated humoral immune responses decrease in aged humans (Cancro et al., 2009; Gibson et al., 2009; Pawelec and Larbi, 2008; Pawelec et al., 2005, 2004; Linton and Dorshkind, 2004). This leads to increased frequency and severity of infectious diseases and reduces the protective effect of vaccinations. Despite intensive research work, many of the basic mechanisms of age-related immune dysfunction have not yet been clarified. However, because the number of elderly people is increasing in the world, identification of the causes and impact of immunosenescence may offer the possibility to improve prevention or delay some infectious diseases and improve the quality of life in our ageing population. Although B cells may suffer from lack of adequate T cell help in ageing (Ademokun et al., 2010), it is now clear that intrinsic changes in B cells also occur and have a significant impact on antibody production. Indeed, with ageing, in the B cell lineage there are reduction in the functional capacities of B cells and their progenitors, changes in the sizes of different subsets and shift in the diversity and clonotypic composition of the antigen-responsive repertoire (Cancro et al., 2009).

A memory B cell subpopulation lacking CD27 and IgD expression and  $IgG^+$  is expanded in elderly people (Colonna Romano et al., 2009). This result, although without statistical significance has also been reported by others (Frasca et al., 2010b). So, the question remains: Where do these  $IgG^+IgD^-CD27^-$  B cells come from and why are increased in healthy elderly? One hypothesis is that these cells could be senescent memory B cells that have down-modulated their expression of CD27, as demonstrated for T cells against chronic antigenic stimuli, e.g. herpetic viruses that fill the immunological space with exhausted antigen-specific CD8 memory T cells (Appay et al., 2002; Pawelec et al., 2004, 2005; Akbar and Fletcher, 2005). This could be also the case in the B cell compartment. On the other hand, it has been demonstrated that chronic stimulation of the immune system, as in patients with lupus (Anolik et al., 2004), is related to the expansion of these cells and that the amount of double negative B cells correlates positively with the clinical manifestations of SLE (Wei et al., 2007). Increased  $IgD^-CD27^-$  DN B cells has been also described in healthy subjects challenged with RSV (Sanz et al., 2008), and this feature may confirm the important role of the chronic antigenic load. So, the expansion of these memory B cell populations might be the manifestation of a physiologic modification time-related (elderly people) or a pathologic deregulation (SLE patients) of the immune system. Furthermore, it is known that elderly produce increased levels of antibodies to autologous antigens often accompanied by autoimmune phenomena (Candore et al., 2007, 1997; Banerjee et al., 2002) and are less able to make high-affinity antibodies to foreign antigens. Indeed, increased  $CD5^+$  B lymphocytes, that, as known, play a key role as producers of autoantibodies (Dalloul, 2009; Youinou et al., 1988), have been demonstrated in old humans (Weksler, 2000) and mice

(Alter-Wolf et al., 2009b), in lupus mouse models (Zhong et al., 2009) and in human rheumatoid arthritis (Nakiri et al., 2007). However in our previous paper (Colonna Romano et al., 2003) we found an age-related decrease of CD5<sup>+</sup> B cells, The intriguing aspect is that, in our elderly population, there is an increase of the IgD<sup>-</sup>CD27<sup>-</sup>DN B cells, and one hypothesis might be that these cells could be responsible for production of autoantibodies or cytokines that lead to an imbalance of the mechanism controlling the immune response against self antigens. Accordingly to their deregulatory feature, IgD<sup>-</sup>CD27<sup>-</sup> B cell were found increased also in hairy cell leukemia patients (Forconi et al., 2004).

Another hypothesis about the origin of these memory CD27<sup>-</sup> B cells is that supported by Wei et al. (2007). They suggested that these cells fail to go through a productive germinal center reaction. So they might develop outside the germinal center in extrafollicular reactions capable of supporting somatic hypermutation and isotype switching in a CD40-independent manner. In agreement, it has been demonstrated that ageing negatively affects germinal center formation in secondary lymphoid tissues (Frasca et al., 2005). So it could be postulated that the increase of the double negative memory B cell population observed in the elderly, but also in successfully aged centenarians, is due to an atypical formation of these cells in extrafollicular reactions. In particular DN B cells obtained from healthy elderly people, show a lower rate of somatic hypermutation (manuscript in preparation), so the reduction of the rate of mutation might be due to a total disconnected generation of these cells, in elderly, from either germinal centers or T cell help. In view of the lack of CD27 expression and the low rate of somatic mutation in IgG<sup>+</sup>CD27<sup>-</sup> B cells, here shown and reported by others (Anolik et al., 2004; Fecteau et al., 2006), it has been hypothesized that IgG<sup>+</sup>CD27<sup>-</sup> B cells might be a first wave of memory B cells (Blink et al., 2005; Inamine et al., 2005) or a pool of short-lived memory B cells (Dorner and Radbruch, 2005), in contrast to the IgG<sup>+</sup>CD27<sup>+</sup> B cells that could be more related to long-lived memory B cells. Alternatively DN B cells might represent activated follicular cells that initiate germinal center reaction, after receiving early CD154-mediated T cell help, but fail to progress through this pathway. This could explain their failure to acquire CD27 and their low rate of somatic hypermutation as compared with classical CD27<sup>+</sup> memory B lymphocytes (Fecteau et al., 2006; our manuscript in preparation).

Data on centenarian offspring support the hypothesis of a “familiar youth” of the immune system, due to their favorable genetic background, that can be a big advantage both to fight the main age-related diseases and to properly respond to vaccinations. In particular, the reservoir of naïve B cell might be one of the causes that make centenarian offspring able to keep fighting off new infections, hence prolonging their life (Colonna Romano et al., 2010). So, on the whole, naïve B cells are well preserved in centenarian offspring, suggesting a good bone marrow cell reservoir. This is an interesting observation, as it has been recently reviewed (Cancro et al., 2009) the bone marrow ability to generate B cells is impaired with age. This could be the reason why in these subjects there are less IgD/CD27 double negative B cells than their age-related counterpart that are not genetically advantaged. Becoming centenarians, their immune system, that allowed them to reach longevity, also proceeds versus exhaustion.

We also show that in old patients affected by Alzheimer's disease, the DN B cells are not increased as in their age-matched controls. The explanation of this is not obvious, but others have reported the decrease of effector CD8<sup>+</sup>CD28<sup>-</sup> T cells in AD patients (Speciale et al., 2007). Together these data support the hypothesis that DN B cells might be exhausted/terminally differentiated B memory cells. In fact they behave as CD8<sup>+</sup>CD28<sup>-</sup> T cells, i.e. are increased in elderly and centenarians, not increased in centenarian offspring (manuscript in preparation) and in AD patients (Speciale et al., 2007). The reduction on total B cells and the increased expres-

sion of the chemokine receptor CCR5 after stimulation with Aβ<sub>42</sub> on B cells from AD patients, suggest us the participation of B cells in the complex cellular interactions active in AD patients (Pellicanò et al., 2010). Most of studies on frailty have focused on T cells, Semba et al. (2005) have demonstrated that the increase of CD8 T cells that lack CD28 is related to frailty, the same has been reported in the impaired response to vaccines (Weng et al., 2009). Moreover De Fanis et al. (2008) have related the amount of CCR5<sup>+</sup> T cells with frailty, and suggest that this correlation might be due to the proinflammatory properties of these cells and the well known link between frailty and chronic inflammation. On the other side it has been demonstrated that B cell repertoire diversity decreases in the elderly with poor health status and this is, to the best of our knowledge the sole report on frailty and B cells in the elderly.

As it is known that memory and naïve B cells produce different cytokines and express different Toll-like receptors, they might have a regulatory role in the chronic infections against viruses and bacteria also producing cytokines and chemokines. This suggests that the immune-inflammation is also related to the B cell branch of the immune system in aged people (Pellicanò et al., 2010; Pasare and Medzhitob, 2005; Duddy et al., 2004; Schultze et al., 1999; Pistoia, 1997). So further studies are necessary to evaluate whether DN B cells in elderly might have some immunomodulatory role as cytokines production.

All together data obtained evaluating DN B cells in healthy elderly, centenarians, centenarian offspring and AD patients might be relevant in the field of immunosenescence and in studies of successful and unsuccessful ageing.

Hence it is important to study the complex process of ageing of the immune system and characterize the changes in both innate and adaptive immunity in order to elaborate better vaccination strategies for the prevention of infectious diseases in the elderly. So, B cell subset changes, associated also to their different regulatory role, could represent a hallmark of successful or unsuccessful ageing and could be used as a biomarker of human life span, potentially useful for the evaluation of anti-ageing treatment to improve the quality of life of the growing elderly population.

## Acknowledgements

Original work discussed in the present review was supported by the Italian Ministry of Education, University and Research grant (Does parental longevity impact on the health ageing of their offspring? An immunological and genetic study) to C. Caruso. SB is PhD student of Pathobiology PhD course (directed by C.C.) at Palermo University and this paper is submitted in partial fulfillment of the requirement for his PhD degree.

## References

- Ademokun, A., Wu, Y., Dunn-Walters, D., 2010. The ageing B cell population: composition and function. *Biogerontology* 11, 125–137.
- Agematsu, K., Hokibara, S., Nagumo, H., Komiyama, A., 2000. CD27: a memory B-cell marker. *Immunol. Today* 21, 204–206.
- Agrawal, S., Gupta, S., 2010. TLR1/2, TLR7, and TLR9 signals directly activate peripheral blood naïve and memory B cell subsets to produce cytokines, chemokines, and hematopoietic growth factors. *J. Clin. Immunol.*, doi:10.1007/s10875-010-9456-8.
- Akbar, A.N., Fletcher, J.M., 2005. Memory T cell homeostasis and senescence during ageing. *Curr. Opin. Immunol.* 17, 480–485.
- Almanzar, G., Schwaiger, S., Jenewein, B., Keller, M., Grubeck-Loebenstien, B., Würzner, R., Schönitzer, D., 2004. IFN-gamma production by CMV-specific CD8<sup>+</sup> T cells is high in elderly donors. *Exp. Gerontol.* 39, 863–865, author reply 867–868.
- Alter-Wolf, S., Blomberg, B.B., Riley, R.L., 2009a. Deviation of the B cell pathway in senescent mice is associated with reduced surrogate light chain expression and altered immature B cell generation, phenotype, and light chain expression. *J. Immunol.* 1 (182), 138–147.
- Alter-Wolf, S., Blomberg, B.B., Riley, R.L., 2009b. Old mice retain bone marrow B1 progenitors, but lose B2 precursors, and exhibit altered immature B cell phenotype and light chain usage. *Mech. Ageing Dev.* 130, 401–408.

- Anolik, J.A., Barnard, J., Cappione, A., Pugh-Bernard, A.E., Felgar, R.A., Looney, R.J., Sanz, I., 2004. Rituximab improves peripheral B cell abnormalities in human systemic lupus erythematosus. *Arthritis Rheum.* 40, 3580–3590.
- Appay, V., Dunbar, P.R., Callan, M., Klennerman, P., Gillespie, G.M.A., Papagno, L., Ogg, G.S., King, A., Lechner, F., Spina, C.A., Little, S., Havlir, D.V., Richman, D.D., Gruener, N., Pape, G., Waters, A., Easterbrook, P., Salio, M., Cerundolo, V., McMichael, A.J., Rowland-Jones, S.L., 2002. Memory CD8<sup>+</sup> T cells vary in differentiation phenotype in different persistent virus infections. *Nat. Med.* 8, 379–385.
- Appay, V., Sauce, D., Prelog, M., 2010. The role of the thymus in immunosenescence: lessons from the study of thymectomized individuals. *Ageing* 2, 78–81.
- Balistreri, C.R., Candore, G., Listi, F., Fazio, T., Gangi, S., Incalcaterra, E., Caruso, M., Vecchi, M.L., Lio, D., Caruso, C., 2007. Role of TLR4 polymorphisms in inflammatory responses: implications for unsuccessful ageing. *Ann. N. Y. Acad. Sci.* 1119, 203–207.
- Balistreri, C.R., Candore, G., Caruso, M., Incalcaterra, E., Franceschi, C., Caruso, C., 2008. Role of polymorphisms of CC-chemokine receptor-5 gene in acute myocardial infarction and biological implications for longevity. *Haematologica* 93, 637–638.
- Banerjee, M., Mehr, R., Belevolsky, A., Spencer, J., Dunn-Walters, D.K., 2002. Age- and tissue-specific differences in human germinal center B cell selection revealed by analysis of IgV<sub>H</sub> gene hypermutation and lineages trees. *Eur. J. Immunol.* 32, 1947–1957.
- Black, S.J.W., van der Loo, M.R., Loken, M.R., Herzberg, L.A., 1978. Expression of IgD by murine lymphocytes: loss of surface IgD indicates maturation of memory B cells. *J. Exp. Med.* 147, 984–996.
- Blink, E.J., Light, A., Kallies, A., Nutt, S.L., Hodgkin, P.D., Tarlinton, D.M., 2005. Early appearance of germinal center-derived memory B cells and plasma cells in blood after primary immunization. *J. Exp. Med.* 201, 545–554.
- Breitbart, E., Wang, X., Leka, L.S., Dallal, G.E., Meydani, S.N., Stollar, B.D., 2002. Altered memory B-cell homeostasis in human ageing. *J. Gerontol. A: Biol. Sci. Med. Sci.* 57, B304–B311.
- Britschgi, M., Wyss-Coray, T., 2007. Systemic and acquired immune responses in Alzheimer's disease. *Int. Rev. Neurobiol.* 82, 205–233.
- Bruunsgaard, H., Andersen-Ranberg, K., Hjelmberg, J.B., Pedersen, B.K., Jeune, B., 2003. Elevated levels of tumor necrosis factor alpha and mortality in centenarians. *Am. J. Med.* 115, 278–283.
- Bulati, M., Pellicanò, M., Vasto, S., Colonna Romano, G., 2008. Understanding ageing: biomedical and bioengineering approaches, the immunologic view. *Immun. Ageing* 5, 9, doi:10.1186/1742-4933-5-9.
- Cancro, M.P., Hao, Y., Scholz, J.L., Riley, R.L., Frasca, D., Dunn-Walters, D.K., et al., 2009. B cells and ageing: molecules and mechanisms. *Trends Immunol.* 30, 313–318.
- Candore, G., Di Lorenzo, G., Mansueto, P., Melluso, M., Fradà, G., Li Vecchi, M., Esposito Pellitteri, M., Drago, A., Di Salvo, A., Caruso, C., 1997. Prevalence of organ-specific and non organ-specific autoantibodies in healthy centenarians. *Mech. Ageing Dev.* 94, 183–190.
- Candore, G., Campagna, A.M., Cuppari, I., Di Carlo, D., Mineo, C., Caruso, C., 2007. Genetic control of immune response in carriers of the 8.1 ancestral haplotype: correlation with levels of IgG subclasses: its relevance in the pathogenesis of autoimmune diseases. *Ann. N. Y. Acad. Sci.* 1110, 151–158.
- Candore, G., Balistreri, C.R., Colonna Romano, G., Grimaldi, M.P., Lio, D., Listi, F., Scola, L., Vasto, S., Caruso, C., 2008. Immunosenescence and anti-immunosenescence therapies: the case of probiotics. *Rejuven. Res.* 11, 425–432.
- Cevenini, E., Caruso, C., Candore, G., Capri, M., Nuzzo, D., Duro, G., Rizzo, C., Colonna Romano, G., Lio, D., Di Carlo, D., Palmas, M.G., Scurti, M., Pini, E., Franceschi, C., Vasto, S., 2010. Age-related inflammation: the contribution of different organs, tissues and systems. how to face it for therapeutic approaches. *Curr. Pharm. Des.* 16, 609–618.
- Chong, Y., Ikematsu, H., Yamaji, K., Nishimura, M., Kashiwagi, S., Hayashi, J., 2003. Age-related accumulation of Ig V(H) gene somatic mutations in peripheral B cells from aged humans. *Clin. Exp. Immunol.* 133, 59–66.
- Chong, Y., Ikematsu, H., Yamaji, K., Nishimura, M., Nabeshima, S., Kashiwagi, S., Hayashi, J., 2005. CD27<sup>+</sup> (memory) B cells decrease and apoptosis-resistant CD27<sup>-</sup> (naïve) B cells increase in aged humans: implication for age-related peripheral B cell developmental disturbances. *Int. Immunol.* 17, 383–390.
- Colonna Romano, G., Cossarizza, A., Aquino, A., Scialabba, G., Bulati, M., Lio, D., Candore, G., Di Lorenzo, G., Fradà, G., Caruso, C., 2002. Age- and gender-related values of lymphocyte subsets in subjects from northern and southern Italy. *Arch. Gerontol. Geriatr. (Suppl)* 8, 99–107.
- Colonna Romano, G., Bulati, M., Aquino, A., Scialabba, G., Candore, G., Lio, D., Motta, M., Malaguarnera, M., Caruso, C., 2003. B cells in the aged: CD27, CD5 and CD40 expression. *Mech. Ageing Dev.* 124, 389–393.
- Colonna Romano, G., Bulati, M., Aquino, A., Pellicanò, M., Vitello, S., Lio, D., Candore, G., Caruso, C., 2009. A double-negative (IgD<sup>-</sup>CD27<sup>-</sup>) B cell population is increased in the peripheral blood of elderly people. *Mech. Ageing Dev.* 130, 81–690.
- Colonna Romano, G., Buffa, S., Bulati, M., Vasto, S., Pellicanò, M., Candore, G., Lio, D., Caruso, C., 2010. B cell compartment in centenarian offspring and old people. *Curr. Pharm. Des.* 16, 604–608.
- Cossarizza, A., Ortolani, C., Paganelli, R., Barbieri, D., Monti, D., Sansoni, P., et al., 1996. CD45 isoforms expression on CD4<sup>+</sup> and CD8<sup>+</sup> T cells throughout life, from newborns to centenarians: implications for T cell memory. *Mech. Ageing Dev.* 86, 173–195.
- Dalloul, A., 2009. CD5: a safeguard against autoimmunity and a shield for cancer cells. *Autoimmun. Rev.* 8, 349–353.
- De Fani, U., Wang, G.C., Fedarko, N.S., Walston, J.D., Casolaro, V., Leng, S.X., 2008. T-lymphocytes expressing CC chemokine receptor-5 are increased in frail older adults. *J. Am. Geriatr. Soc.* 56, 904–908.
- Derhovanessian, E., Maier, A.B., Beck, R., Jahn, G., Hähnel, K., Slagboom, P.E., de Craen, A.J., Westendorp, R.G., Pawelec, G., 2010. Hallmark features of immunosenescence are absent in familial longevity. *J. Immunol.* 185, 4618–4624.
- Di Bona, D., Plaia, A., Vasto, S., Cavallone, L., Lescai, F., Franceschi, C., Licastro, F., Colonna Romano, G., Lio, D., Candore, G., Caruso, C., 2008. Association between the interleukin-1beta polymorphisms and Alzheimer's disease: a systematic review and meta-analysis. *Brain Res. Rev.* 59, 155–163.
- Di Bona, D., Candore, G., Franceschi, C., Licastro, F., Colonna Romano, G., Cammà, C., Lio, D., Caruso, C., 2009. Systematic review by meta-analyses on the possible role of TNF-alpha polymorphisms in association with Alzheimer's disease. *Brain Res. Rev.* 61, 60–68.
- Dorner, T., Radbruch, A., 2005. Selecting B cells and plasma cells to memory. *J. Exp. Med.* 201, 497–499.
- Duddy, M.E., Alter, A., Bar-Or, A., 2004. Distinct profiles of human B cell effector cytokines. *J. Immunol.* 172, 3422–3427.
- Ershler, W.B., Keller, E.T., 2000. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. *Ann. Rev. Med.* 51, 245–270.
- Fagnoni, F.F., Vescovini, R., Mazzola, M., Bologna, G., Nigro, E., Lavagetto, G., Franceschi, C., Passeri, M., Sansoni, P., 1996. Expansion of cytotoxic CD8<sup>+</sup>CD28<sup>-</sup> T cells in healthy ageing people, including centenarians. *Immunology* 88 (4), 501–507.
- Faria, A.M., Ficker, S.M., Speziali, E., Menezes, J.S., Stransky, B., Verdolin, B.A., Lahmann, W.M., Rodrigues, V.S., Vaz, N.M., 1998. Aging and immunoglobulin isotype patterns in oral tolerance. *Braz. J. Med. Biol. Res.* 31, 35–48.
- Faria, A.M., de Moraes, S.M., de Freitas, L.H., Speziali, E., Soares, T.F., Figueiredo-Neves, S.P., Vitelli-Avelar, D.M., Martins, M.A., Barbosa, K.V., Soares, E.B., Sathler-Avelar, R., Peruhype-Magalhaes, V., Cardoso, G.M., Comin, F., Teixeira, R., Eloi-Santos, S.M., Queiroz, D.M., Correa-Oliveira, R., Bauer, M.E., Teixeira-Carvalho, A., Martins-Filho, O.A., 2008. Variation rhythms of lymphocyte subsets during healthy ageing. *Neuroimmunomodulation* 15, 365–379.
- Fecteau, J.F., Néron, S., 2003. CD40 stimulation of human peripheral B lymphocytes: distinct response from naïve and memory cells. *J. Immunol.* 171, 4621–4629.
- Fecteau, J.F., Coté, G., Néron, S., 2006. A new memory CD27<sup>+</sup> IgG<sup>+</sup> B cell population in peripheral blood expressing VH genes with low frequency of somatic mutation. *J. Immunol.* 177, 3728–3736.
- Forconi, F., Sahota, S.S., Raspadori, D., Ippoliti, M., Babbage, G., Lauria, F., Stevenson, F.K., 2004. Hairy cell leukemia: at the crossroad of somatic mutation and isotype switch. *Blood* 104, 3312–3317.
- Franceschi, C., Monti, D., Sansoni, P., Cossarizza, A., 1995. The immunology of exceptional individuals: the lessons of centenarian. *Immunol. Today* 16, 12–16.
- Franceschi, C., Bonafe, M., Valensin, S., 2000a. Human immunosenescence: the prevailing of innate immunity, the failing of clonotypic immunity, and the filling of immunological space. *Vaccine* 18, 1717–1720.
- Franceschi, C., Valensin, S., Bonafè, M., Paolisso, G., Yashin, A.I., Monti, D., De Benedictis, G., 2000b. The network and the remodeling theories of ageing: historical background and new perspectives. *Exp. Gerontol.* 35, 879–896.
- Franceschi, C., Bonafe, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., De Benedictis, G., 2000c. Inflamm-aging: An evolutionary perspective on immunosenescence. *Ann. N. Y. Acad. Sci.* 908, 244–254.
- Franceschi, C., Olivieri, F., Marchegiani, F., Cardelli, M., Cavallone, L., Capri, M., Salvilioli, S., Valensin, S., De Benedictis, G., Di Iorio, A., Caruso, C., Paolisso, G., Monti, D., 2005. Genes involved in immune response/inflammation, IGF1/insulin pathway and response to oxidative stress play a major role in the genetics of human longevity: the lesson of centenarians. *Mech. Ageing Dev.* 126, 351–361.
- Franceschi, C., Capri, M., Monti, D., Giunta, S., Olivieri, F., Sevini, F., Panourgia, M.P., et al., 2007. Inflammaging and anti-inflammaging: a systemic perspective of ageing and longevity emerged from studies in humans. *Mech. Ageing Dev.* 128, 92–105.
- Frasca, D., Van der Put, E., Riley, R.L., Blomberg, B.B., 2004. Reduced Ig class switch in aged mice correlates with decreased E47 and activation-induced cytidine deaminase. *J. Immunol.* 172, 2155–2162.
- Frasca, D., Riley, R.L., Blomberg, B.B., 2005. Humoral immune response and B-cell functions including immunoglobulin class switch are downregulated in aged mice and humans. *Sem. Immunol.* 17, 378–384.
- Frasca, D., Riley, R.L., Blomberg, B.B., 2007. Aging murine B cells have decreased class switch induced by anti-CD40 or BAFF. *Exp. Gerontol.* 42, 192–203.
- Frasca, D., Landin, A.M., Lechner, S.C., Ryan, J.G., Schwartz, R., Riley, R.L., Blomberg, B.B., 2008. Ageing down-regulates the transcription factor E2A, activation-induced cytidine deaminase, and Ig class switch in human B cells. *J. Immunol.* 180, 5283–5290.
- Frasca, D., Diaz, A., Romero, M., Landin, A.M., Phillips, M., Lechner, S.C., Ryan, J.G., Blomberg, B.B., 2010a. Intrinsic defects in B cell response to seasonal influenza vaccination in elderly humans. *Vaccine*, doi:10.1016/j.vaccine.2010.10.023.
- Frasca, D., Diaz, A., Romero, M., Landin, A.M., Blomberg, B.B., 2010b. Age effects on B cells and humoral immunity in humans. *Ageing Res Rev.* doi:10.1016/j.arr.2010.08.004.
- Gao, H.M., Hong, J.S., 2008. Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. *Trends Immunol.* 29, 357–365.
- Gardner, E.M., Nogales, E.W., Nogusa, S., Murasko, D.M., 2006. Age-related changes in the immune response to influenza vaccination in a racially diverse, healthy elderly population. *Vaccine* 24, 1609–1614.
- Genton, B., D'Acremont, V., Furrer, H.J., Hatz, C., Louis, L., 2006. Hepatitis A vaccines and the elderly. *Travel. Med. Infect. Dis.* 4, 303–312.

- Gibson, K.L., Wu, Y.C., Barnett, Y., Duggan, O., Vaughan, R., Kondeatis, E., Nilsson, B.O., Wikby, A., Kipling, D., Dunn-Walters, D.K., 2009. B-cell diversity decreases in old age and is correlated with poor health status. *Ageing Cell* 8, 18–25.
- Ginaldi, L., Loreto, M.F., Corsi, M.P., Modesti, M., De Martinis, M., 2001. Immunosenescence and infectious diseases. *Microb. Infect.* 3, 851–857.
- Ginaldi, L., Di Benedetto, M.C., De Martinis, M., 2005. Osteoporosis, inflammation and ageing. *Immun. Ageing* 2, 14.
- Globerson, A., Effros, R.B., 2000. Ageing of lymphocytes and lymphocytes in the aged. *Immunol. Today* 21, 515–521.
- Gruver, A.L., Hudson, L.L., Sempowski, G.D., 2007. Immunosenescence of ageing. *J. Pathol.* 211, 144–156.
- Guerretaz, L.M., Johnson, S.A., Cambier, J.C., 2008. Acquired hematopoietic stem cell defects determine B-cell repertoire changes associated with aging. *Proc. Natl. Acad. Sci. U.S.A.* 105, 11898–11902.
- Gupta, S., Su, H., Bi, R., Agrawal, S., Gollapudi, S., 2005. Life and death of lymphocytes: a role in immunosenescence. *Immun. Ageing* 2, 12, doi:10.1186/1742-4933-2-12.
- Harris, D.P., Haynes, L., Sayles, P.C., Duso, D.K., Eaton, S.M., Lepak, N.M., et al., 2000. Reciprocal regulation of polarized cytokine production by effector B and T cell. *Nat. Immunol.* 1, 475–482.
- Huppert, F.A., Solomou, W., O'Connor, S., Morgan, K., Sussams, P., Brayne, C., 1998. Ageing and lymphocyte subpopulations: whole-blood analysis of immune markers in a large population sample of healthy elderly individuals. *Exp. Gerontol.* 33, 593–600.
- Inamine, A., Takahashi, Y., Baba, N., Miyake, K., Tokuhisa, T., Takemori, T., Abe, R., 2005. Two waves of memory B cell generation in the primary immune response. *Int. Immunol.* 17, 581–589.
- Johnson, K.M., Owen, K., Witte, P.L., 2002. Aging and developmental transitions in the B cell lineage. *Int. Immunol.* 14, 1313–1323.
- Kline, G.H., Hayden, T.A., Klinman, N.R., 1999. B cell maintenance in aged mice reflects both increased B cell longevity and decreased B cell generation. *J. Immunol.* 162, 3342–3349.
- Klein, U., Rajewsky, K., Küppers, R., 1998. Human immunoglobulin (Ig)M\*<sup>+</sup>IgD<sup>+</sup> peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. *J. Exp. Med.* 188, 1679–1689.
- Koga, T., McGhee, J.R., Kato, H., Kato, R., Kiyono, K., Fujihashi, K., 2000. Evidence for early aging in the mucosal immune system. *J. Immunol.* 165, 5352–5359.
- Kolar, G.R., Mehta, D., Wilson, P.C., Capra, J.D., 2006. Diversity of the Ig repertoire is maintained with age in spite of reduced germinal centre cells in human tonsil lymphoid tissue. *Scand. J. Immunol.* 64, 314–324.
- Kolibab, K., Smithson, S.L., Rabquer, B., Khuder, S., Westerink, M.A., 2005. Immune response to pneumococcal polysaccharides 4 and 14 in elderly and young adults: analysis of the variable heavy chain repertoire. *Infect. Immun.* 73, 7465–7476.
- Kriangkum, J., Taylor, B.J., Treon, S.P., Mant, M.J., Belch, A.R., Pilarski, L.M., 2004. Clonotypic IgH VDJ sequence analysis in Waldenström's macroglobulinemia suggests an unusual B cell origin and expansion of polyclonal B cells in peripheral blood. *Blood* 104, 2134–2142.
- Kruetzmann, S., Rosado, M.M., Weber, H., Germing, U., Tournilhac, O., Peter, H.H., Berner, R., Peters, A., Boehm, T., Plebani, A., et al., 2003. Human immunoglobulin M memory B cells controlling *Streptococcus pneumoniae* infections are generated in the spleen. *J. Exp. Med.* 197, 939–945.
- LeMaout, J., Delassus, S., Dyal, R., Nikolić-Zugić, J., Kourilsky, P., Weksler, M.E., 1997. Clonal expansions of B lymphocytes in old mice. *J. Immunol.* 159, 3866–3874.
- Libby, P., 2002. Inflammation in atherosclerosis. *Nature* 420, 868–874.
- Linton, P.J., Dorshkind, K., 2004. Age-related changes in lymphocyte development and function. *Nat. Immunol.* 5, 133–139.
- Lio, D., Candore, G., Crivello, A., Scola, L., Colonna Romano, G., Cavallone, L., Hoffmann, E., Caruso, M., Licastro, F., Caldarera, C.M., Branzi, A., Franceschi, C., Caruso, C., 2004. Opposite effects of interleukin 10 common gene polymorphisms in cardiovascular diseases and in successful ageing: genetic background of male centenarians is protective against coronary heart disease. *J. Med. Genet.* 41, 790–794.
- Listì, F., Candore, G., Modica, M.A., Russo, M.A., Di Lorenzo, G., Esposito-Pellitteri, M., Colonna Romano, G., Aquino, A., Bulati, M., Lio, D., Franceschi, C., Caruso, C., 2006. A study of serum immunoglobulin levels in elderly persons that provides new insights into B cell immunosenescence. *Ann. N. Y. Acad. Sci.* 1089, 487–495.
- Lougaris, V., Badolato, R., Ferrari, S., Plebani, A., 2005. Hyper immunoglobulin M syndrome due to CD40 deficiency: clinical, molecular, and immunological features. *Immunol. Rev.* 203, 48–66.
- Manz, R.A., Thiel, A., Radbruch, A., 1997. Lifetime of plasma cells in the bone marrow. *Nature* 388, 133–134.
- Martin, F., Chan, A.C., 2006. B cell immunobiology in disease: evolving concepts from the clinic. *Annu. Rev. Immunol.* 24, 467–496.
- McElhane, J.E., Effros, R., 2009. Immunosenescence: what does it mean to health outcomes in older adults? *Curr. Opin. Immunol.* 21, 418–424.
- McKenna, R.W., Washington, L.T., Aquino, D.B., Picker, L.J., Kroft, S.H., 2001. Immunophenotypic analysis of hematogones (B-lymphocyte precursors) in 662 consecutive bone marrow specimens by 4-color flow cytometry. *Blood* 98, 2498–2507.
- Miller, J.P., Allman, D., 2003. The decline in B lymphopoiesis in aged mice reflects loss of very early B-lineage precursors. *J. Immunol.* 171, 2326–2330.
- Miller, J.P., Cancro, M.P., 2007. B cell and ageing: balancing the homeostatic equation. *Exp. Gerontol.* 42, 396–399.
- Minges Wols, H.A., Johnson, K.M., Ippolito, J.A., Birjandi, S.Z., Su, Y., Le, P.T., Witte, P.L., 2009. Migration of immature and mature B cells in the aged microenvironment. *Immunology* 129, 278–290.
- Monsonogo, A., Zota, V., Karni, A., Krieger, J.I., Bar-Or, A., Bitan, G., Budson, A.E., Sperling, R., Selkoe, D.J., Weiner, H.L., 2003. Increased T cell reactivity to amyloid beta protein in older humans and patients with Alzheimers disease. *J. Clin. Invest.* 112, 415–422.
- Murasko, D.M., Bernstein, E.D., Gardner, E.M., Gross, P., Munk, G., Dran, S., et al., 2002. Role of humoral and cell-mediated immunity in protection from influenza disease after immunization of healthy elderly. *Exp. Gerontol.* 37, 427–439.
- Nakiri, Y., Minowa, K., Suzuki, J., Mitsuo, A., Amano, H., Morimoto, S., Tokano, Y., Takasaki, Y., 2007. Expression of CD22 on peripheral B cells in patients with rheumatoid arthritis: relation to CD5-positive B cells. *Clin. Rheumatol.* 26, 1721–1723.
- Nicholson, K.G., Kent, J., Hammersley, V., Cancio, E., 1997. Acute viral infections of upper respiratory tract in elderly people living in the community: comparative, prospective, population based study of disease burden. *BMJ* 315, 1060–1064.
- Nicoletti, C., Borghesi-Nicoletti, C., Yang, X.H., Schulze, D.H., Cerny, J., 1991. Repertoire diversity of antibody response to bacterial antigens in aged mice. II. Phosphorylcholine antibody in young and aged mice differ in both VH/VL gene repertoire and in specificity. *J. Immunol.* 147, 2750–2755.
- Nicoletti, C., Yang, X., Cerny, J., 1993. Repertoire diversity of antibody response to bacterial antigens in aged mice. III. Phosphorylcholine antibody from young and aged mice differ in structure and protective activity against infection with *Streptococcus pneumoniae*. *J. Immunol.* 150, 543–549.
- Obukhanych, T.V., Nussenzweig, M.C., 2006. T-independent type II immune responses generate memory B cells. *J. Exp. Med.* 203, 305–310.
- O'Mahony, L., Holland, J., Jackson, J., Feighery, C., Hennessy, T.P., Mealy, K., 1998. Quantitative intracellular cytokine measurement: age-related changes in proinflammatory cytokine production. *Clin. Exp. Immunol.* 113, 213–219.
- Ouyang, Q., Wagner, W.M., Voehringer, D., Wikby, A., Klatt, T., Walter, S., Müller, C.A., Pircher, H., Pawelec, G., 2003. Age-associated accumulation of CMV-specific CD8<sup>+</sup> T cells expressing the inhibitory killer cell lectin-like receptor G1 (KLRG1). *Exp. Gerontol.* 38, 911–920.
- Paganelli, R., Quinti, I., Fagiolo, U., Cossarizza, A., Ortolani, C., Guerra, E., Sansoni, P., Pucillo, L.P., Scala, E., Cozzi, E., Bertollo, L., Monti, D., Franceschi, C., 1992. Changes in circulating B cells and immunoglobulin classes and subclasses in a healthy aged population. *Clin. Exp. Immunol.* 90, 351–354.
- Pasare, C., Medzhitob, R., 2005. Control of B-cell responses by Toll-like receptors. *Nature* 438, 364–368.
- Pawelec, G., Barnett, Y., Forsey, R., Frasca, D., Globerson, A., McLeod, J., et al., 2002. T cells and ageing. *Front. Biosci.* 7, d1056–d1183.
- Pawelec, G., Akbar, A.N., Caruso, C., Effros, R., Grubeck-Loebenstein, B., Wikby, A., 2004. Is immunosenescence infectious? *Trends Immunol.* 25, 406–410.
- Pawelec, G., Akbar, A.N., Caruso, C., Solana, R., Grubeck-Loebenstein, B., Wikby, A., 2005. Human immunosenescence: is it infectious? *Immunol. Rev.* 20, 257–268.
- Pawelec, G., Larbi, A., 2008. Immunity and ageing in man: Annual Review 2006/2007. *Exp. Gerontol.* 43, 34–38.
- Pellicanò, M., Bulati, M., Buffa, S., Barbagallo, M., Di Prima, A., Misiano, G., Picone, P., Di Carlo, M., Nuzzo, D., Candore, G., Vasto, S., Lio, D., Caruso, C., Colonna Romano, G., 2010. Systemic immune response in Alzheimer's disease: in vitro mononuclear cell activation and cytokine production. *J. Alzheimers Dis.* 21, 181–192.
- Pes, G.M., Lio, D., Carru, C., Deiana, L., Baggio, G., Franceschi, C., Ferrucci, L., Oliveri, F., Scola, L., Crivello, A., Candore, G., Colonna Romano, G., Caruso, C., 2004. Association between longevity and cytokine gene polymorphisms. A study in Sardinian centenarians. *Ageing Clin. Exp. Res.* 16, 244–248.
- Pillai, S., Cariappa, A., Moran, S.T., 2004. Positive selection and lineage commitment during peripheral B lymphocyte development. *Immunol. Rev.* 197, 206–218.
- Pillai, S., Cariappa, A., Moran, S.T., 2005. Marginal zone B cells. *Annu. Rev. Immunol.* 23, 161–196.
- Pistoia, V., 1997. Production of cytokines by human B cells in health and disease. *Immunol. Today* 18, 343–350.
- Qi, H., Egen, J.G., Huang, A.Y.C., Germain, R.N., 2006. Extrafollicular activation of lymph node B cells by antigen-bearing dendritic cells. *Science* 312, 1672–1676.
- Riley, R.L., Kruger, M.G., Elia, J., 1991. B cell precursors are decreased in senescent BALB/c mice, but retain normal mitotic activity in vivo and in vitro. *Clin. Immunol. Immunopathol.* 59, 301–313.
- Rogers, J., Lubner-Narod, J., Styren, S.D., Civin, W.H., 1998. Expression of immune system associated antigens by cells of the human central nervous system: relationship to the pathology of Alzheimer's disease. *Neurobiol. Ageing* 9, 339–349.
- Rossi, M.I., Yokota, T., Medina, K.L., Garrett, K.P., Comp, P.C., Schipul Jr., A.H., et al., 2003. B lymphopoiesis is active throughout human life, but there are developmental age-related changes. *Blood* 101, 576–584.
- Sansoni, P., Cossarizza, A., Brianti, V., Fagnoni, F., Snelli, G., Monti, D., et al., 1993. Lymphocyte subsets and natural killer cell activity in healthy old people and centenarians. *Blood* 82, 2767–2773.
- Sanz, I., Anolik, J.H., Looney, R.J., 2007. B cell depletion therapy in autoimmune disease. *Front. Biosci.* 12, 2546–2567.
- Sanz, I., Wei, C., Lee, F.E., Anolik, J., 2008. Phenotypic and functional heterogeneity of human memory B cells. *Semin. Immunol.* 20, 67–82.
- Schenkein, J.G., Park, S., Nahm, M.H., 2008. Pneumococcal vaccination in older adults induces antibodies with low opsonic capacity and reduced antibody potency. *Vaccine* 26, 5521–5526.
- Schultze, J.L., Michalak, S., Lowne, J., Wong, A., Gilleece, M.H., Gribben, J.G., et al., 1999. Human non-germinal center B cell interleukin (IL)-12 production is pri-

- marily regulated by T cell signals CD40 ligand, interferon gamma, and IL-10: role of B cells in the maintenance of T cell responses. *J. Exp. Med.* 189, 1–12.
- Semba, R.D., Margolick, J.B., Leng, S., Walston, J., Ricks, M.O., Fried, L.P., 2005. T cell subsets and mortality in older community-dwelling women. *Exp. Gerontol.* 40, 81–87.
- Shi, Y., Yamazaki, T., Okubo, Y., Uehara, Y., Sugane, K., Agematsu, K., 2005. Regulation of aged humoral immune defense against pneumococcal bacteria by IgM memory B cell. *J. Immunol.* 175, 3262–3267.
- Shriner, A.K., Smithson, S.L., Rabquer, B., Khuder, S., Westerink, M.A., 2006. Analysis of the young and elderly variable gene repertoire in response to pneumococcal polysaccharides using a reconstituted SCID mouse model. *Vaccine* 24, 7159–7166.
- Smithson, S.L., Kolibab, K., Shriner, A.K., Srivastava, N., Khuder, S., Westerink, M.A., 2005. Immune response to pneumococcal polysaccharides 4 and 14 in elderly and young adults: analysis of the variable light chain repertoire. *Infect. Immun.* 73, 7477–7484.
- Speciale, L., Calabrese, E., Saresella, M., Tinelli, C., Mariani, C., Sanvito, L., Longhi, R., Ferrante, P., 2007. Lymphocyte subset patterns and cytokine production in Alzheimer's disease patients. *Neurobiol. Ageing* 28, 1163–1169.
- Spencer, J., Perry, M.E., Dunn-Walters, D.K., 1998. Human marginal-zone B cells. *Immunol. Today* 19, 421–426.
- Speziali, E., Santiago, A.F., Fernandes, R.M., Vaz, N.M., Menezes, J.S., Faria, A.M.C., 2009. Specific immune responses but not basal functions of B and T cells are impaired in aged mice. *Cell. Immunol.* 256, 1–5.
- Tangye, S.G., Hodgkin, P.D., 2004. Divide and conquer: the importance of cell division in regulating B cell responses. *Immunology* 112, 509–520.
- Terry, D.F., Wilcox, M.A., McCormick, M.A., Perls, T.T., 2004a. Cardiovascular disease delay in centenarian offspring. *J. Gerontol. A: Biol. Sci. Med. Sci.* 59, 385–389.
- Terry, D.F., Wilcox, M.A., McCormick, M.A., Pennington, J.Y., Schoenhofen, E.A., Andersen, S.L., et al., 2004b. Cardiovascular advantages among the offspring of centenarians. *J. Am. Geriatr. Soc.* 52, 2074–2076.
- Toellner, K.M., Jenkinson, W.E., Taylor, D.R., Khan, M., Sze, D.M.Y., Sansom, D.M., Vinuesa, C.G., MacLennan, I.C.M., 2002. Low-level hypermutation in T cell-independent germinal centers compared with high mutation rates associated with T cell-dependent germinal centers. *J. Exp. Med.* 195, 383–389.
- van Baarle, D., Tsegaye, A., Miedema, F., Akbar, A., 2005. Significance of senescence for virus-specific memory T cell responses: rapid ageing during chronic stimulation of the immune system. *Immunol. Lett.* 97, 19–29.
- Vasto, S., Caruso, C., 2004. Immunity & Ageing: a new journal looking at ageing from an immunological point of view. *Immun. Ageing* 29, 1–11.
- Vasto, S., Colonna-Romano, G., Larbi, A., Wikby, A., Caruso, C., Pawelec, G., 2007. Role of persistent CMV infection in configuring T cell immunity in the elderly. *Immun. Ageing* 4, 2.
- Veneri, D., Ortolani, R., Franchini, M., Tridente, G., Pizzolo, G., Vella, A., 2009. Expression of CD27 and CD23 on peripheral blood B lymphocytes in humans of different ages. *Blood Transfus.* 7, 29–34.
- Wack, A., Cossarizza, A., Heltai, S., Barbieri, D., D'Addato, S., Franceschi, C., Dellabona, P., Casorati, G., 1998. Age-related modifications of the human alpha beta T cell repertoire due to different clonal expansions in the CD4<sup>+</sup> and CD8<sup>+</sup> subsets. *Int. Immunol.* 10, 1281–1288.
- Wang, X., Stollar, B.D., 1999. Immunoglobulin VH gene expression in human aging. *Clin. Immunol.* 93, 132–142.
- Wei, C., Anolik, J., Cappione, A., Zheng, B., Pugh-Bernard, A., Brooks, J., Lee, E.H., Milner, E.C.B., Sanz, I., 2007. A new population of cells lacking expression of CD27 represents a notable component of the B cell memory compartment in systemic lupus erythematosus. *J. Immunol.* 178, 6624–6633.
- Weinberger, B., Herndler-Brandstetter, D., Schwanninger, A., Weiskopf, D., Grubeck-Loebenstein, B., 2008. Biology of immune responses to vaccine in elderly persons. *Clin. Infect. Dis.* 46, 1078–1084.
- Weinberger, B., Weiskopf, D., Grubeck-Loebenstein, B., 2009. Immunology and ageing. In: Halter, J.B., Ouslander, J.G., Tinetti, M.E., et al. (Eds.), *Hazzard's Geriatric Medicine and Gerontology*. 6th edn. McGraw-Hill, Columbus, pp. 23–36.
- Weksler, M.E., Szabo, P., 2000. The effect of age on the B-cell repertoire. *J. Clin. Immunol.* 20, 240–249.
- Weksler, M.E., 2000. Changes in the B-cell repertoire with age. *Vaccine* 18, 1624–1628.
- Weksler, M.E., Relkin, N., Turkenich, R., LaRusse, S., Zhou, L., Szabo, P., 2002. Patients with Alzheimer disease have lower levels of serum anti-amyloid peptide antibodies than healthy elderly individuals. *Exp. Gerontol.* 37, 943–948.
- Weller, S., Faili, A., Garcia, C., Braun, M.C., Le Deist, F., de Saint Basileqq, G., Hermine, O., Fischer, A., Reynaud, C.A., Weill, J.C., 2001. CD40-CD40L independent Ig gene hypermutation suggests a second B cell diversification pathway in humans. *Proc. Natl. Acad. Sci. U.S.A.* 98, 1166–1170.
- Weller, S., Braun, M.C., Tan, B.K., Rosenwald, A., Cordier, C., Conley, M.E., Plebani, A., Kumararatne, D.S., Bonnet, D., Tournilhac, O., et al., 2004. Human blood IgM "memory" B cells are circulating splenic marginal zone B cells Harboring a pre-diversified immunoglobulin repertoire. *Blood* 104, 3647–3654.
- Weller, S., Reynaud, C.A., Weill, J.C., 2005. Splenic marginal zone B cells in humans: where do they mutate their Ig receptor? *Eur. J. Immunol.* 35, 2789–2792.
- Weng, N., Akbar, A.N., Goronzy, J., 2009. CD28-T cells: their role in the age-associated decline of the immune function. *Trends Immunol.* 30, 306–312.
- Wikby, A., Johansson, B., Ferguson, F., Olsson, J., 1994. Age related changes in immune parameters in a very old population of Swedish people: a longitudinal study. *Exp. Gerontol.* 29, 531–541.
- William, J., Euler, C., Christensen, S., Shlomchik, M.J., 2002. Evolution of autoantibody responses via somatic hypermutation outside of germinal centers. *Science* 297, 2066–2070.
- Wolniak, K.L., Shinal, S.M., Waldschmidt, T.J., 2004. The germinal center response. *Crit. Rev. Immunol.* 24, 39–65.
- Wu, Y.C., Kipling, D., Leong, H.S., Martin, V., Ademokun, A.A., Dunn-Walters, D.K., 2010. High throughput immunoglobulin repertoire analysis distinguishes between human IgM memory and switched memory B cell populations. *Blood* 116, 1070–1078.
- Xiao, Y., Hendriks, J., Langerak, P., Jacobs, H., Borst, J., 2004. CD27 is acquired by primed B cells at the centroblast stage and promotes germinal center formation. *J. Immunol.* 172, 7432–7441.
- Yan, J., Greer, J.M., Hull, R., O'Sullivan, J.D., Henderson, R.D., Read, S.J., McCombe, P.A., 2010. The effect of ageing on human lymphocyte subsets: comparison of males and females. *Immun. Ageing* 7, 4.
- Youinou, P., Mackenzie, L., Broker, B.M., Isemberg, D.I., Drogou-Lelong, A., Gentric, A., Lyduard, P.M., 1988. The importance of CD5-positive B cells in nonorgan-specific autoimmune diseases. *Scand. J. Rheumatol.* 76, 243–249.
- Zheng, B., Han, S., Takahashi, Y., Kelsoe, G., 1997. Immunosenscence and germinal center reaction. *Immunol. Rev.* 160, 63–77.
- Zhong, X., Lau, S., Bai, C., Degauque, N., Holodick, N.E., Steven, S.J., Tumang, J., Gao, W., Rothstein, T.L., 2009. A novel subpopulation of B-1 cells is enriched with autoreactivity in normal and lupus-prone mice. *Arthritis Rheum.* 60, 3734–3743.