Immunology 1978 **35** 601

Age-dependent variations of antibody avidity*

G. DORIA, G. D'AGOSTARO & A. PORETTI *CNEN-Euratom Immunogenetics Group, Laboratory of Radiopathology, C.S.N. Casaccia (Rome), Italy*

Received 5 *December* 1977; *accepted for publication* 2 *February* 1978

Summary. Age-dependent variations of antibody avidity were studied in the C3HeB/FeJ mouse. Spleen cells from donors of different ages (10–720 days) were transferred and stimulated with TNP-HRBC in lethally irradiated syngenic recipients. The anti-TNP antibody response of the donor cells was estimated from the number of direct PFC per recipient spleen by the Jerne technique with TNP-SRBC. Avidity of the antibodies secreted by PFC was evaluated from the amount of added TNP-BSA that inhibited 50% of the anti-TNP PFC. Under these experimental conditions allowing the exclusion of any influence of the donor milieu during the immune response, age-dependent variations of the antibody response and avidity could be attributed to changes in the donor spleen cell population.

Avidity was found to increase with the response and to vary parabolically with age. After appropriate correction of the number of PFC to make it independent from age, avidity values were fitted by a multiple curvilinear regression in which the independent variables playing a significant role were the corrected number of PFC in its linear term and the age in its linear and quadratic terms. From comparison of the standard coefficients of this regression, the observed variations of avidity could be attributed in part (82%) to the response and in part (18%) to the age. For any value of response, avidity increased 15-fold from day 10 to reach a maximum at day 110 and then declined 5-fold at the age of 720 days. Heterogeneity of avidity also changed parabolically with age as high avidity classes were present in adulthood and absent at 10 and 720 days.

* Supported by CNEN-Euratom Contract. Publication No. 1485 of the Euratom Biology Division.

Correspondence: Dr G. Doria, CNEN-Euratom Immunogenetics Group, Laboratory of Radiopathology, C.S.N. Casaccia (Rome), Italy.

0019-2805/78/1000-0601$02.00

INTRODUCTION

Immunological competence is known to vary with age. In most animal species the antibody response increases during postnatal development, reaches a maximum in adulthood, and then declines during senescence. These age-dependent variations are determined by changes in the animal milieu and in the cells of the immune system. Although there is little doubt that humoral factors play a role in the development (Murgita & Tomasi, 1975) and decline (Price & Makinodan, 1972b) of immunological competence, the cellular changes and their functional consequences were found to account for most of the variation of immunological competence.

In B lymphocytes of the foetal mouse spleen, membrane-bound Igs appear before the capacity to mount an antibody response (Spear & Edelman, 1974; Rosenberg & Cunningham, 1975). However, immature B lymphocytes can produce specific

antibodies if immunized upon transfer into lethally irradiated recipients enriched in helper T lymphocytes (Chiscon & Golub, 1972; Press & Klinman, 1973; Klinman & Press, 1975). Also the postnatal development of the *in vitro* antibody response of mouse spleen cells is correlated with an increase of a T-cell population highly responsive to PHA and Con A (Spear & Edelman, 1974). Furthermore, the *in vitro* (Mosier & Johnson, 1975) or *in vivo* (Morse, Prescott, Cross, Stashak & Baker, 1976) antibody response to T-dependent or -independent antigens during the first weeks of life is influenced by the presence of suppressor T-cells. Thus, the B-cell reactivity during the development of the immune system is conditioned by T-cell subpopulations. The role of other phenomena intrinsic to the B lymphocyte, such as changes of the Ig receptor density (Scher, Sharrow, Wister, Asofsky & Paul, 1976) and class (Vitetta, Melchers, McWilliams, Lamm, Phillips-Quagliata & Uhr, 1975) or appearance of the C3 receptor (Gelfand, Elfenbein, Frank & Paul, 1974; Sidman & Unanue, 1975), in the expression of the cell immune potential is ill defined. Macrophages seem to have no role in the development of the immunological competence (Rabinowitz, 1976; Fidler, Chiscon & Golub, 1972; Landahl, 1976).

The decline of immunological competence is characterized by several cell changes. Stem cells are not decreased in absolute number but display a lower rate of self-replication and differentiation into haematopoietic colonies (Albright & Makinodan, 1976) and B lymphocytes (Farrar, Loughman & Nordin, 1974) as well as a reduced ability to repair DNA damage after sublethal doses of radiation (Chen, 1974). Macrophages seem unaffected by ageing, as neither phagocytosis (Perkins, 1971) nor the ability to co-operate with T and B cells (Heidrick & Makinodan, 1973) are reduced in old animals. The B-cell population as a whole does not seem to change appreciably with age, as evaluated by the number of cells with Ig receptors and of cells responsive to T-independent antigens and specific mitogens (Makinodan & Adler, 1975) although certain B-cell subpopulations were shown to decrease slightly (Price & Makinodan, 1972a; Kishimoto, Takahama & Mizumachi, 1976; Gerbase-De Lima, Wilkinson, Smith & Walford, 1974). The decline of immune functions during senescence is primarily due to changes in the T-cell component of the immune system (Kishimoto & Yamamura, 1971; Price & Makinodan, 1972a) which have been attributed to thymic involution (Hirokawa & Makinodan, 1975). The reduced mitotic responsiveness to PHA and Con A (Hori, Perkins & Halsall, 1973; Meredith, Gerbase-De Lima & Walford, 1975) suggests a diminution of the helper activity of T cells in the antibody response of old animals. Moreover, the suppressor activity of T cells was found to increase with age (Makinodan, Albright, Good, Peter & Heidrick, 1976; Segre & Segre, 1976a; Goidl, Innes & Weksler, 1976a).

Antibody avidity is influenced by changes in the size and functions of lymphocyte populations, as the B-cell population can produce antibodies of higher or lower affinity upon interaction with helper (Gershon & Paul, 1971) or suppressor (Tada, Taniguchi & Takemori, 1975) T cells, respectively. Since the lymphocyte populations vary with age, the present study was carried out to measure quantitative changes of antibody avidity as a function of age. Spleen cells from mice of different ages (10–720 days) were transferred and stimulated with TNP-HRBC (2,4,6-trinitrophenyl-horse red blood cells), a T-dependent antigen, in lethally irradiated syngenic recipients. The immune response of donor cells against TNP was estimated from the number of direct PFC (plaque-forming cells) per recipient spleen. Avidity of antibodies secreted by PFC was evaluated from the amount of added TNP-BSA (-bovine serum albumin) that inhibited 50% of the anti-TNP PFC. Under these experimental conditions allowing the exclusion of any influence of the donor milieu during the immune response, age-dependent variations of the antibody response and avidity can be attributed to changes in the donor spleen cell population.

MATERIALS AND METHODS

Mice

Inbred C3HeB/FeJ male and female mice were used in all experiments. The mean life span of these animals was about 580 days. The age of the donors varied from 10 to 720 days, that of the recipients was 90 days. In each experiment, donors and recipients were of the same sex.

Antigens

HRBC and SRBC (sheep red blood cells) were prepared from blood in Alsever's solution, purchased from Sclavo (Italy), by repeated washings with PBS

(phosphate-buffered saline). TNP-HRBC were prepared by heavy coupling of TNBS (2,4,6-trinitrobenzensulphonic acid) to HRBC as described by Kettman & Dutton (1970) and used as immunogen. TNP-SRBC were prepared by light coupling of TNBS to SRBC according to Rittenberg & Pratt (1969) and used as test antigen in the Jerne technique (Jerne & Nordin, 1963) to detect direct PFC anti-TNP. The conjugate TNP-BSA (35 mols TNP/mol BSA) was purchased from Calbiochem (U.S.A.) and used in the Jerne technique to inhibit PFC anti-TNP (Doria, Schiaffini, Garavini & Mancini, 1972; Doria, Agarossi, Boraschi & Amendolea, 1977). Amounts of the inhibitor TNP-BSA were expressed in terms of TNP-lysyl residues calculated from spectrophotometric measurements of TNP-BSA solutions in PBS at λ max 348 nm assuming 15,400 as the molar extinction coefficient for the TNP-lysyl residue (Little & Eisen, 1967).

Spleen cell transfer

At the age of 10, 28, 48, 90, 180, 270, 360, 450, 630, or 720 days, 20–60 donors were killed by cervical dislocation and their spleens removed, pooled, and dissociated in Eagle's medium by gentle teasing with a scalpel. The cell suspension was centrifuged twice at 220 *g* for 30 min at 4°. The pellet was resuspended in medium and filtered through a 20 μ nylon mesh. Nucleated cells were counted in a haemocytometer and their concentration adjusted to 96×10^6 cells/ml. The spleen cell suspension was serially diluted with medium in a two-fold manner and then a volume of each suspension was added to an equal volume of PBS containing 1×10^9 TNP-HRBC/ml. One ml aliquots of each final suspension, containing 48, 24, 12, 6, or 3×10^6 spleen cells and 5×10^8 TNP-HRBC, were injected i.v. into eight recipients which had been exposed (whole-body) to 900 rad of *X*-rays 3–4 h prior to the cell transfer. The recipients had been carrier-primed 3 days before irradiation by i.v. injection of 2×10^6 HRBC in 0·2 ml PBS. The transferred spleen cells were restimulated by i.v. injection of 5×10^8 TNP-HRBC in 1 ml PBS 3 days after the first injection of the conjugate. The recipients of each spleen cell dose were usually killed on day 6 after cell transfer, their spleens pooled, suspended in medium, filtered through a 100 μ nylon mesh, and assayed in the Jerne technique for the number of direct PFC and for antibody avidity. In preliminary experiments, recipients were also killed on days 5, 7, 8, and 9 after cell transfer.

Assay for direct anti-TNP PFC

This and the following assay for antibody avidity have been described in detail elsewhere (Doria *et al.*, 1972 and 1977). Briefly, a spleen cell suspension from 5–8 recipients was plated in duplicate dishes with agar containing TNP-SRBC or unconjugated SRBC. Plates were incubated at 37° for 1 h and, after addition of guinea-pig complement, for another 30 min. The mean number of PFC counted in plates with unconjugated SRBC was subtracted from the mean number of PFC in plates with TNP-SRBC to provide the net number of anti-TNP PFC. The magnitude of the anti-TNP response was expressed as the number of direct anti-TNP PFC per recipient spleen.

Assay for antibody avidity

The assay was based on inhibition of direct anti-TNP PFC by soluble TNP-BSA. After the number of anti-TNP PFC per spleen was assessed as described above, the same spleen cell suspension was properly diluted so that an aliquot of it was expected to yield approximately 300 net anti-TNP PFC. This aliquot was plated in duplicate dishes with unconjugated SRBC, or TNP-SRBC, or TNP-SRBC and known amounts of soluble TNP-BSA ranging from 10^{-5} to 10^{-1} mg TNP-lysyl residues. PFC were developed, counted and net numbers of anti-TNP PFC in each dish calculated as above. The percent inhibition of anti-TNP PFC increased as a sigmoid function of the log* amount of inhibitor added. From probit analysis of the data and antilog transformations the ED50 (median effective dose of inhibitor that suppresses 50% of PFC) was calculated by a computer programme. The reciprocal of ED50 (mg^{-1}) was taken as an estimate of avidity; the higher the antibody avidity, the lower the ED50 and the higher its reciprocal. Appropriate controls performed as already described (Doria *et al.*, 1972 and 1977) demonstrated that the avidity estimate was independent of the number of plated anti-TNP PFC and that TNP-BSA was devoid, in the same dose range, of non-specific inhibitory effects on plaque formation which might result from cell toxicity, complement inactivation, or complement consumption by fixation onto complexes of TNP-BSA with anti-TNP antibodies. This method for measuring avidity has been validated by DeLisi (1976) on

* The term log represents $\log_{10}$ if not otherwise specified.

Table 1. Effect of carrier-priming on the anti-TNP antibody response of 48 × 10^6 spleen cells transferred from 90-day-old donors in lethally irradiated recipients

HRBC*	Spleen cells	TNP-HRBC†	Days after spleen cell transfer 5	6	7	8
+	+	+	52‡	226	436	55
−	+	+	7	47	42	32
+	+	−	4	5	4	2
−	+	−	3	7	2	9

(+) injected; (−) non injected.

* 2 × 10^5 HRBC injected 3 days prior to irradiation.

† 5 × 10^8 TNP-HRBC injected immediately and 3 days after irradiation.

‡ anti-TNP PFC (× 10^3)/spleen.

theoretical grounds and by experiments (unpublished) showing that the avidity estimate is independent from the antibody secretion rate.

RESULTS

Preliminary experiments

The spleen cell transfer system used throughout this work was devised on the basis of preliminary experiments.

The need for carrier-priming the recipients to obtain a high anti-TNP response of the transferred cells is illustrated in Table 1. The response of 48 × 10^6 spleen cells from 90-day-old donors was higher in carrier-primed than in -unprimed recipients, showing a carrier effect of 10-fold at the time of the peak response on day 7 after cell transfer. The background response of the same cells without immunization was similar in carrier-primed and -unprimed recipients and comparable to the response of the same recipients given the immunogen but no spleen cells

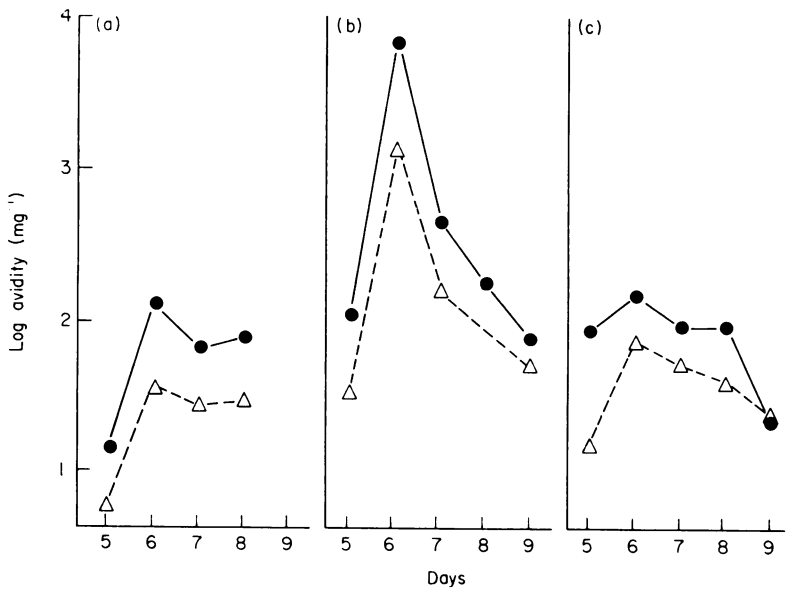

Figure 1. Antibody avidity at different times after the transfer and antigenic stimulation of 12 or 48 × 10^6 spleen cells from donors killed at 25, 90, or 540 days of age. (●), 48 × 10^6; (△), 12 × 10^6. (a) 25-day-old donors; (b) 90-day-old donors; (c) 540-day-old donors.

(data not shown). In another experiment, 12×10^6 spleen cells from 90-day-old donors were immunized in recipients either carrier-unprimed or -primed by 2×10^4, 2×10^5, 2×10^6, or 2×10^7 HRBC. The dose of 2×10^6 provided the maximum carrier effect and was chosen for the subsequent experiments.

The anti-TNP response of spleen cells immunized in optimally carrier-primed recipients was found maximum on day 7 after cell transfer, regardless of the spleen cell dose (12 or 48×10^6) or the donor age (25, 90, or 540 days). The avidity values shown in Fig. 1 indicate that antibody avidity increases to a maximum on day 6 after cell transfer and then declines, regardless of the spleen cell dose and the donor age. Furthermore, larger spleen cell doses yield higher avidity values regardless of the donor age. Finally, at each cell dose the peak avidity varies parabolically with the donor age. Based on these results it was decided for the subsequent experiments to kill recipients on day 6 after the cell transfer.

Relationship between antibody response and number of transferred spleen cells

The log PFC increased linearly with the log spleen cells. As exemplified in Fig. 2 the regression changed with age in that the response of higher spleen cell doses ($12–48 \times 10^6$) varied parabolically with age ($10 < 90 > 450$ days), but the response of lower doses ($3–6 \times 10^6$) varied in a different manner ($90 < 450 > 10$ days). Thus, changes of both intercept and slope of the regression hindered unbiased estimates of age-dependent variations of the antibody response. However, valuable information could be gathered from comparison of the regression slopes. Figure 3 shows that the regression coefficient was less than 1 at the age of 10 days, about 1 at 28 days, and usually greater than 1 at 90 and subsequent days. These variations suggest that the antibody response

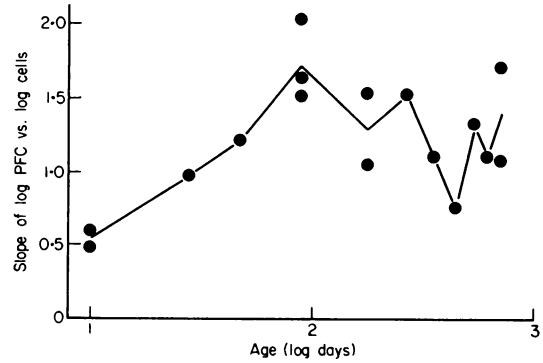

Figure 3. Variations of the slope of the regression log PFC versus log spleen cells as a function of the donor age.

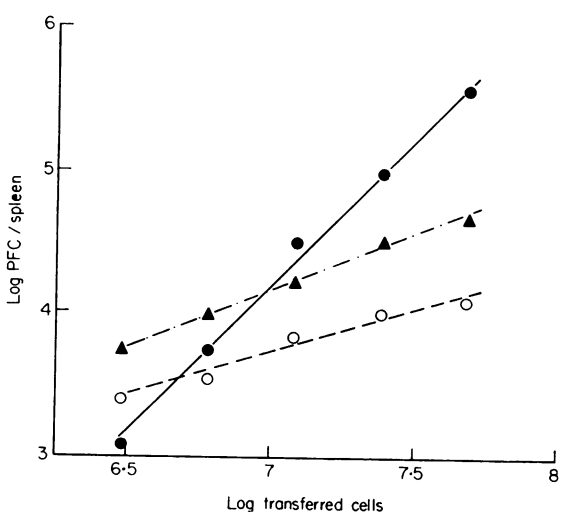

Figure 2. Relationship between antibody response and number of spleen cells transferred from donors killed at 25, 90, or 540 days of age. (○), 10 days (slope 0·6); (●), 90 days (slope 2·0); (▲), 450 days (slope 0·8).

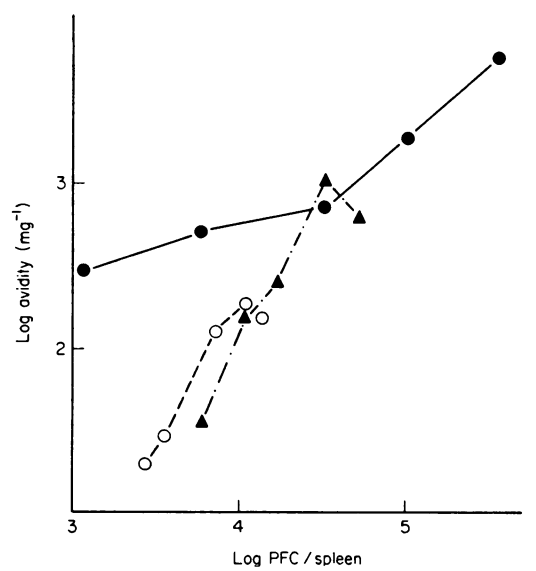

Figure 4. Log avidity versus log PFC at different donor ages. (○), 10 days; (●), 90 days; (▲), 450 days.

of spleen cells from donors of different ages results from different types and degrees (Mosier & Coppleson, 1968) of cell interactions.

Relationship between antibody avidity and response

Since both the avidity (Fig. 1) and the response (Fig. 2) depend on the spleen cell dose, then avidity may also be a function of the response. Fig. 4 shows that avidity does vary with the number of PFC. It follows that comparisons of antibody avidities of different ages should be made at the same level of response. Also these data, as those of Fig. 1, indicate that avidity changes in a parabolic manner with age.

Mathematical model for evaluating avidity as a function of the antibody response and age

The results presented above indicate that avidity increases with the response (Fig. 4) and varies parabolically with age (Figs 1 and 4). It appears that the variations of avidity with age could be described by a multiple curvilinear regression as follows:

$$A = k_1P + k_2P^2 + k_3D + k_4D^2 + k_5DP + C$$

where A = log avidity, P = log PFC, D = log days, C = intercept, k_{1-5} = coefficients.

The model requires that the response (P) be independent of age (D), a condition not fulfilled by the data (Fig. 2). This difficulty was overcome by transforming log PFC in another variable independent from age. For each age, the transformation

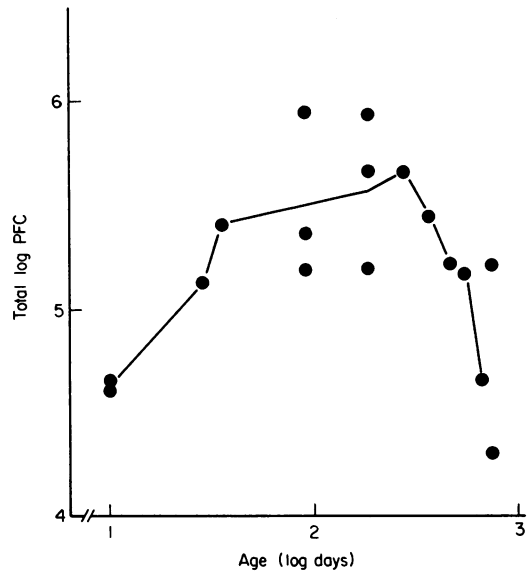

Figure 5. Total log PFC produced by 3–48 × 10^6 spleen cells as a function of the donor age.

consisted in dividing log PFC by the integral of the regression of log PFC on log spleen cells in the interval between 3 and 48 × 10^6 transferred cells. The integral, designated (log PFC)$_t$, is a variable that changes parabolically with age (Fig. 5) and could therefore be used to transform log PFC. The transformation log PFC/(log PFC)$_t$ was efficient because, as shown in Fig. 6 the transformed variable, designated (log PFC)$_w$, is clearly independent of

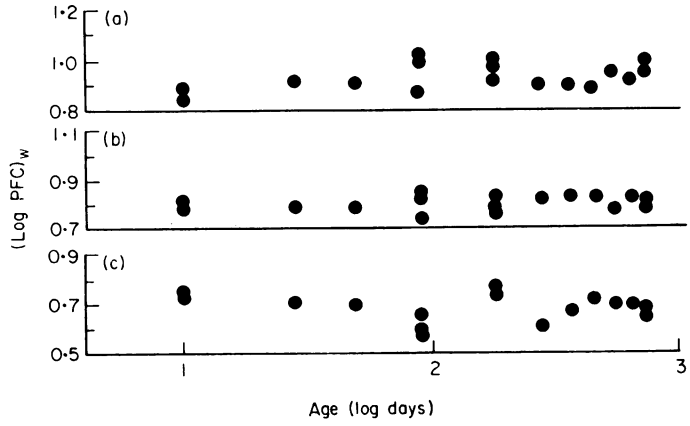

Figure 6. Independence of (log PFC)$_w$ from age at different spleen cell doses. (a) 48 × 10^6 spleen cells; (b) 12 × 10^6 spleen cells (c) 3 × 10^6 spleen cells.

Table 2. Multiple curvilinear regression. $A = 3{\cdot}115\ P + 4{\cdot}368\ D - 1{\cdot}068\ D^2 - 3{\cdot}709$

Variable	Standard coefficient	Relative contribution
P_w	0·542	0·82
D	4·066	0·18
D^2	−3·946	

Standard coefficient = Regression coefficient × $\frac{\text{Coefficient standard deviation}}{\text{Total standard deviation}}$.

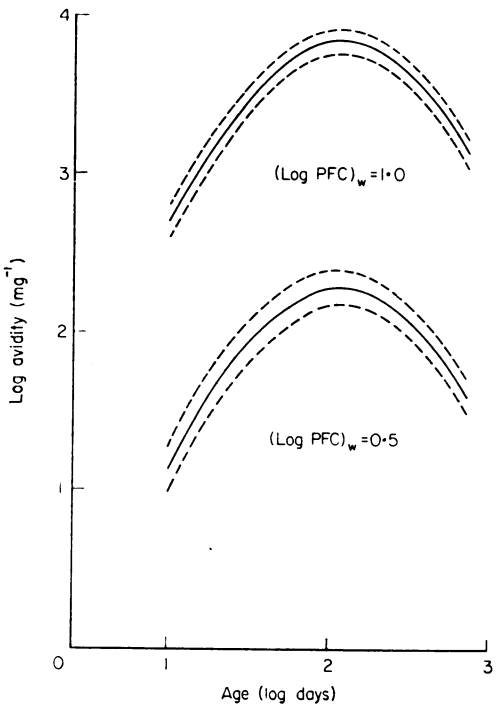

Figure 7. Variations of avidity with age at two levels of $(\log PFC)_w$. The continuous lines were generated by the equation reported in Table 2. Dotted lines represent 95% confidence limits.

age. Thus, log PFC was replaced by $(\log PFC)_w$ and, therefore, P by P_w in the mathematical model.

Since some terms of the complete equation of the mathematical model might be redundant, the significance of the contribution of each variable to the multiple regression was evaluated by stepwise analysis (Steel & Torrie, 1960). Briefly, each variable was introduced stepwise and its contribution considered as significant if it reduced the sum of squares. Only P_w, D and D^2 were found significant variables and used in the working form of the equation as reported in Table 2. The regression coefficients were transformed in standard coefficients, expressed in units of standard deviation, and then compared to assess the relative contribution of the response and age to the variations of avidity. From Table 2, 82% of the observed variations of avidity can be attributed to the response while 18% to age. Thus, for any value of response (P_w), the equation generates a curve describing the variations of avidity due only to age (Fig. 7): avidity increased 15-fold from day 10 to reach a maximum at day 110 and then declined 5-fold at the age of 720 days.

Heterogeneity of antibody avidity

The distribution of a PFC population in avidity classes can be calculated from the inhibition curve of anti-TNP PFC by TNP-BSA, as described in Fig. 8. The histograms of the central part refer to approximately the same antibody response (iso-PFC) of spleen cell populations from 10-, 180-, or 720-day-old donors. Avidity is higher and more heterogeneous at 180 days than at 10 and 720 days. The histograms of the left and right parts of Fig. 8 refer to maximum and minimum levels of avidity and show in both cases that avidity at 180 days is again higher and more heterogeneous than that at 10 and 720 days. Thus, the rise and fall of avidity with age as described by the application of the mathematical model (Fig. 7) is confirmed also at extreme values of avidity. In all cases, high values were characterized by the presence of higher avidity classes and greater heterogeneity.

DISCUSSION

The size of the anti-TNP antibody response of the donor spleen cells was found to increase to a maximum at 90–180 days and to decrease subsequently (Fig. 5). This result is in line with previous findings from *in vivo* and *in vitro* studies on the ontogeny (Playfair, 1968; Chiscon & Golub, 1972; Fidler *et al.*, 1972; Spear & Edelman, 1974; Rosenberg & Cunningham, 1975; Mosier & Johnson, 1975; Rabinowitz, 1976; Hardy, Mozes & Danon, 1976; Morse *et al.*, 1976; Landahl, 1976) and senescence (Albright & Makinodan, 1968; Kishimoto, Tsuyuchi & Yamamura, 1969; Kishimoto & Yamamura,

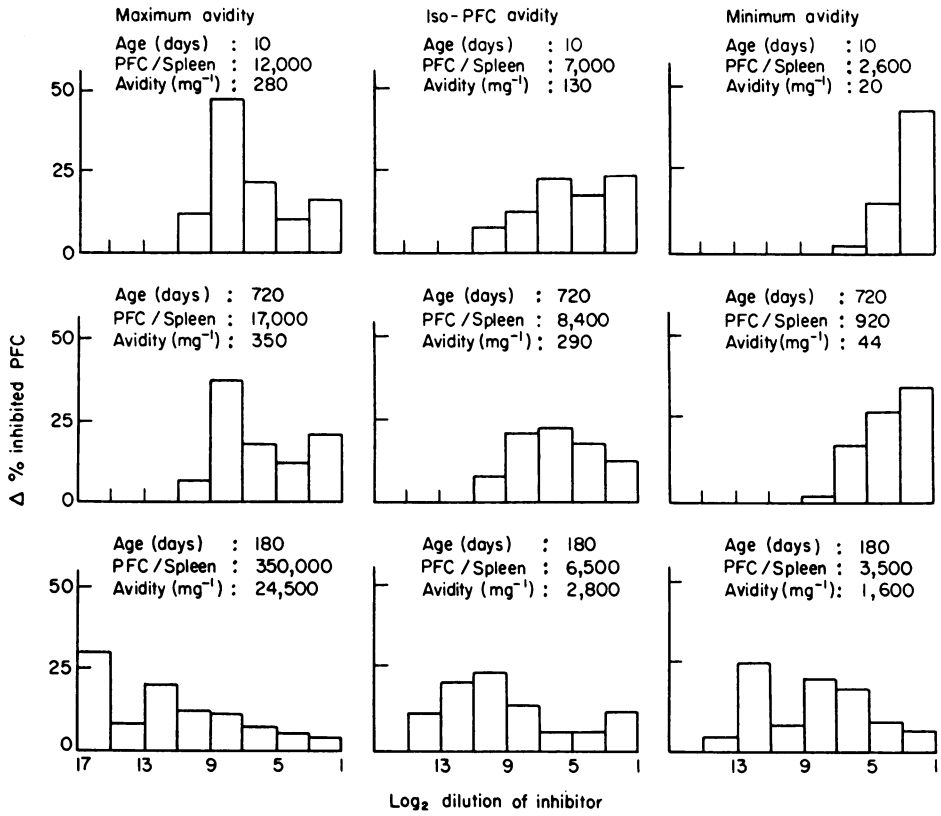

Figure 8. Heterogeneity of antibody avidity. The histogram distributions of avidities were calculated from the plaque inhibition curves. The height of each column represents the difference between the percent inhibitions of anti-TNP PFC at two consecutive $\log_2$ dilutions of inhibitor. Higher avidity classes correspond to higher dilutions of inhibitor. The histograms of the central part represent the avidity distributions at approximately the same level of response (iso-PFC) of spleen cells from donors of different ages. The histograms of the left and right parts represent the heterogeneity at the maximum and minimum levels of avidity.

1971; Price & Makinodan, 1972a, b; Heidrick & Makinodan, 1973; Kishimoto *et al.*, 1976; Makinodan *et al.*, 1976; Segre & Segre, 1976a, b) of the immune response. In addition, it was found that the number of PFC per transferred cell varies with the age of the spleen cell donors, as represented in Fig. 3 by the age-dependent variations of the coefficient of the regression log PFC versus log spleen cells. Since coefficient values greater than 1 have been attributed to cell interactions (Mosier & Coppleson, 1968), the value being an estimate of the number of interacting cell populations (Coppleson & Michie, 1966), the data of Fig. 3 suggest the existence of different types and degrees of cell interactions at different ages. At 90 days, the regression coefficient was about 2, implying a positive interaction probably between hapten-specific B cells and carrier-specific helper T cells (Klinman, 1972; North & Feinstein, 1973). Similar results were found by others for the primary response (Gregory & Lajtha, 1968) to SRBC and the secondary response (Celada, 1967) to human serum albumin in adoptive transfers of spleen cells from adult mice. At the age of 10 days, the regression coefficient was less than 1, a value compatible with a negative cell interaction. As a matter of fact, suppressor T cells have been found abundant in the spleen of 2-week-old mice (Mosier & Johnson, 1975). At the ages of 28 and 48 days, regression coefficients were close to 1 suggesting a balance between help and suppression. Thus, from the present data and other findings on the response to T-independent antigens (Morse *et*

al., 1976) the development of the immunological potential in the mouse spleen at the age of 10–90 days seems to be characterized by reduction of suppressor cells paralleled by increase of helper cells. At the age of 180–720 days, the values of the regression coefficients were less than 2 but usually greater than 1. This decline is compatible with the following possibilities: (a) decrease in size and reactivity of the helper T-cell population; (b) increase of the suppressor T-cell population. The reduced responsiveness of lymphocytes from old mice to PHA and Con A (Hori *et al.*, 1973; Meredith *et al.*, 1975) favours mechanism (a). On the other hand, mechanism (b) is supported by experiments showing increase of suppressive activity in the spleens of old mice (Makinodan *et al.*, 1976; Segre & Segre, 1976a; Goidl *et al.*, 1976a). The two possibilities are not mutually exclusive.

Antibody avidity displayed parabolic changes with age, reaching an interpolated maximum value at 110 days which was 15-fold greater than that at 10 days and 5-fold greater than that at 720 days (Fig. 7). Heterogeneity of avidity also changed parabolically with age, regardless of the experimental conditions allowing the expression of minimum, intermediate, or maximum avidity levels (Fig. 8). At each of these levels, the parabolic pattern was characterized by the presence in adulthood (180 days) of high avidity classes which were missing at the ages of 10 and 720 days. These findings are in agreement with previous indications from experiments in which avidity also was measured by plaque inhibition (Goidl & Siskind, 1974; Goidl, Klass & Siskind, 1976b; Marshall-Clarke & Playfair, 1975; Goidl *et al.*, 1976a). In the present study, however, the age-dependent variations of avidity were evaluated at the same level of antibody response by a procedure based on the partitioning of the total variation in components attributable to response or age. The necessity of sorting out the variation of avidity due only to age is stressed by the evidence (Table 2) that 82% of the total variation was due to the antibody response. This should not be surprising since it has been shown that antibody avidity is positively correlated with the size of the response (Celada, Schmidt & Strom, 1969).

It has been found repeatedly that antibody avidity depends on interactions between T and B cells, avidity being increased by helper cells (Gershon & Paul, 1971; Doria *et al.*, 1977) and decreased by suppressor cells (Takemori & Tada, 1974; Tada *et al.*, 1975). The age-dependent changes of the T-cell population, which were considered above to explain the various degrees of cell interactions (Fig. 3), may also account for the observed changes of avidity. Thus, the increase of avidity and of its heterogeneity from 10 to 90 days of age could reflect a decrease of suppressor and an increase of helper functions. The successive fall of avidity could be attributed to a decline of the number and reactivity of helper cells as well as to an enlargement of the suppressor cell population. In addition, alterations of the B-cell population could contribute to age-dependent variations of avidity. Indeed, antigen-specific B cells with receptors of higher affinity might appear in the spleen in greater numbers as the development of the immunological competence proceeds with time (Goidl & Siskind, 1974; Klinman & Press, 1975). This possibility, however, seems to be contradicted by the concomitant presence of both low and high affinity B cells in the mouse embryo (D'Eustachio & Edelman, 1975). During senescence, the slight decrease of the mitotic responsiveness of B lymphocytes to LPS (Kishimoto *et al.*, 1976; Gerbase-De Lima *et al.*, 1974) might be taken as suggestive evidence for the appearance of functional impairment of these cells in the ageing mouse. Alterations of the biosynthetic machinery could result from several mechanisms, such as error accumulation (Orgel, 1963), codon restriction (Streheler, Hirsch, Gusseck, Johnson & Bich, 1971), exhaustion of redundant messages (Medvedev, 1972), transcription errors (Von Hahn, 1973), and affect significantly the affinity of specific antibodies synthesized by B cells.

The observed age-dependent variations in antibody response and avidity were interpreted mainly as the result of changes in cell interactions among the transferred spleen cells. The variation of donor T-cell subpopulations, which is well known to occur with age (see Introduction), was indicated as a likely explanation of the data. This interpretation need not conflict with the fact that all experiments were done in carrier-primed recipients to obtain high anti-TNP responses. As a matter of fact, the recipient help did not abrogate the difference in response of the same numbers of spleen cells from donors of different age. It cannot be excluded that B cells from donors of a different age have different ability to co-operate with the recipient helper T cells. This possibility, however, is not supported by previous observations (Chiscon & Golub, 1972;

Makinodan & Adler, 1975). It should be pointed out that the effects of cell interactions on antibody avidity may be mediated to some extent by antibodies. During the immune response, the selection of high affinity receptor cells could be influenced by the antigen and antibody concentrations: the antibody competes with the cells for antigen and provides a selective drive in favour of higher affinity cells which can capture free antigen in low concentration and be stimulated. Since spleen cells from donors of different age yielded PFC responses of different magnitude, the higher avidity associated with higher response might have resulted, at least in part, from the greater selective pressure exerted by the higher antibody concentration.

REFERENCES

ALBRIGHT J.F. & MAKINODAN T. (1968) Growth and senescence of antibody-forming cells. *J. Cell. Comp. Physiol.* **67** (Suppl. 1), 185.

ALBRIGHT J.W. & MAKINODAN T. (1976) Decline in the growth potential of spleen-colonizing bone marrow stem cells of long-lived aging mice. *J. exp. Med.* **144,** 1204.

CELADA F. (1967) Quantitative studies of the adoptive immunological memory in mice. II. Linear transmission of cellular memory. *J. exp. Med.* **125,** 199.

CELADA F., SCHMIDT D. & STROM R. (1969) Determination of avidity of anti-albumin antibodies in the mouse. Influence of the number of cells transferred on the quality of the secondary adoptive response. *Immunology,* **17,** 189.

CHEN M.G. (1974) Impaired Elkind recovery in hematopoietic colony-forming cells of aged mice. *Proc. Soc. exp. Biol. Med.* **145,** 1181.

CHISCON M.O. & GOLUB E.S. (1972) Functional development of the interacting cells in the immune response. I. Development of T and B cell function. *J. Immunol.* **108,** 1379.

COPPLESON L.W. & MICHIE D. (1966) A quantitative study of the chorioallantoic membrane reaction in the chick embryo. *Proc. R. Soc. Biol.* **163,** 555.

DELISI C. (1976) Hemolytic plaque inhibition: the physical chemical limits on its use as affinity assay. *J. Immunol.* **117,** 2249.

D'EUSTACHIO P. & EDELMAN G.E. (1975) Frequency and avidity of specific antigen-binding cells in developing mice. *J. exp. Med.* **142,** 1078.

DORIA G., AGAROSSI G., BORASCHI D. & AMENDOLEA M.A. (1977) Effect of carrier priming on antibody avidity in the *in vivo* and *in vitro* immune response. *Immunology,* **32,** 539.

DORIA G., SCHIAFFINI G., GARAVINI M. & MANCINI C. (1972) The rise and fall of antibody avidity at the level of single immunocytes. *J. Immunol.* **108,** 54.

FARRAR J.J., LOUGHMAN B.E. & NORDIN A.A. (1974) Lymphopoietic potential of bone marrow cells from aged mice: comparison of the cellular constituents of bone marrow from young and aged mice. *J. Immunol.* **112,** 1244.

FIDLER J.M., CHISCON M.O. & GOLUB E.S. (1972) Functional development of the interacting cells in the immune response. II. Development of immunocompetence to heterologous erythrocytes *in vitro*. *J. Immunol.* **109,** 136.

GELFAND M.C., ELFENBEIN G.J., FRANK M.M. & PAUL W.E. (1974) Ontogeny of B-lymphocytes. II. Relative rates of appearance of lymphocytes bearing surface immunoglobulin and complement receptors. *J. exp. Med.* **139,** 1125.

GERBASE-DE LIMA M., WILKINSON J., SMITH G.S. & WALFORD R.L. (1974) Age-related decline in thymic-independent immune function in a long-lived mouse strain. *J. Gerontol.* **29,** 261.

GERSHON R.K. & PAUL W.E. (1971) Effect of thymus-derived lymphocytes on amount and affinity of antihapten antibody. *J. Immunol.* **106,** 872.

GOIDL E.A., INNES J.B. & WEKSLER M.E. (1976a) Immunological studies of aging. II. Loss of IgG and high avidity plaque-forming cells and increased suppressor cell activity in aging mice. *J. exp. Med.* **144,** 1037.

GOIDL E.A., KLASS J. & SISKIND G.W. (1976b) Ontogeny of B-lymphocyte function. II. Ability of endotoxin to increase the heterogeneity of affinity of the immune response of B-lymphocytes from fetal mice. *J. exp. Med.* **143,** 1503.

GOIDL E.A. & SISKIND G.W. (1974) Ontogeny of B-lymphocyte function. I. Restricted heterogeneity of the antibody response of B lymphocytes from neonatal and fetal mice. *J. exp. Med.* **140,** 1285.

GREGORY C.J. & LAJTHA L.G. (1968) Kinetic study of the production of antibody-forming cells from their precursors. *Nature,* **218,** 1079.

HARDY B., MOZES E. & DANON D. (1976) Comparison of the immune potential of newborn mice to T-dependent and T-independent synthetic polypeptides. *Immunology,* **30,** 261.

HEIDRICK M.L. & MAKINODAN T. (1973) Presence of impairment of humoral immunity in nonadherent spleen cells of old mice. *J. Immunol.* **111,** 1502.

HIROKAWA K. & MAKINODAN T. (1975) Thymic involution: effect on T cell differentiation. *J. Immunol.* **114,** 1659.

HORI Y., PERKINS E.H. & HALSALL M.K. (1973) Decline in phytohemagglutinin responsiveness of spleen cells from aging mice. *Proc. Soc. exp. Biol. Med.* **144,** 48.

JERNE N.K. & NORDIN A.A. (1963) Plaque formation in agar by single antibody producing cells. *Science,* **140,** 405.

KETTMAN J. & DUTTON R.W. (1970) An *in vitro* primary immune response to 2,4,6-trinitrophenyl substituted erythrocytes: response against carrier and hapten. *J. Immunol.* **104,** 1558.

KISHIMOTO S., TAKAHAMA T. & MIZUMACHI H. (1976) *In vitro* immune response to the 2,4,6-trinitrophenyl determinant in aged C57Bl/6J mice: changes in the humoral response to avidity for the TNP determinant and responsiveness to LPS effect with aging. *J. Immunol.* **116,** 294.

KISHIMOTO S., TSUYUCHI I. & YAMAMURA Y. (1969) Immune response in aged mice. *Clin. exp. Immunol.* **5,** 525.

KISHIMOTO S. & YAMAMURA Y. (1971) Immune response in aged mice: changes of antibody-forming cell precursors and antigen-reactive cells with age. *Clin. exp. Immunol.* **8**, 957.

KLINMAN N.R. (1972) The mechanism of antigenic stimulation of primary and secondary clonal precursor cell. *J. exp. Med.* **136**, 241.

KLINMAN N.R. & PRESS J.L. (1975) The characterization of the B-cell repertoire specific for the 2,4-dinitrophenyl and 2,4,6-trinitrophenyl determinants in neonatal Balb/c mice. *J. exp. Med.* **141**, 1133.

LANDAHL C.A. (1976) Ontogeny of adherent cells. I. Distribution and ontogeny of A cells participating in the response to sheep erythrocytes *in vitro*. *Europ. J. Immunol.* **6**, 130.

LITTLE J.R. & EISEN H.N. (1967) In: *Methods in Immunology and Immunochemistry* (ed. by C. A. Williams and M. W. Chase), Volume 1, p. 131. Academic Press, London.

MAKINODAN T. & ADLER W. (1975) The effects of aging on the differentiation and proliferation potentials of cells of the immune system. *Fed. Proc.* **34**, 153.

MAKINODAN T., ALBRIGHT J.W., GOOD P.I., PETER C.P. & HEIDRICK M.L. (1976) Reduced humoral immune activity in long-lived old mice: an approach to elucidating its mechanisms. *Immunology*, **31**, 903.

MARSHALL-CLARKE S. & PLAYFAIR J.H.L. (1975) Age-dependent changes in the relative affinity of anti-dinitrophenyl antibodies in mice. *Immunology*, **29**, 477.

MEDVEDEV Z.A. (1972) Repetition of molecular-genetic information as a possible factor in evolutionary changes of lifespan. *Exp. Gerontol.* **7**, 227.

MEREDITH P., GERBASE-DE LIMA M. & WALFORD R.L. (1975) Age-related changes in PHA : ConA stimulatory ratios of cells from spleens of a long-lived mouse strain. *Exp. Gerontol.* **10**, 247.

MORSE H.C., PRESCOTT B., CROSS S.S., STASHAK P.W. & BAKER P.J. (1976) Regulation of the antibody response to type III pneumococcal polysaccharide. V. Ontogeny of factors influencing the magnitude of the plaque-forming cell response. *J. Immunol.* **116**, 279.

MOSIER D.E. & COPPLESON L.W. (1968) A three-cell interaction required for the induction of the primary immune response *in vitro*. *Proc. nat. Acad. Sci.* **61**, 542.

MOSIER D.E. & JOHNSON B.M. (1975) Ontogeny of mouse lymphocyte function. II. Development of the ability to produce antibody is modulated by T lymphocytes. *J. exp. Med.* **141**, 216,

MURGITA R.A. & TOMASI T.B. (1975) Suppression of the immune response by α-fetoprotein. I. The effect of mouse α-fetoprotein on the primary and secondary antibody response. *J. exp. Med.* **141**, 269.

NORTH J.R. & FEINSTEIN A. (1973) Analysis of anti-hapten foci in repopulated mouse spleens. *Europ. J. Immunol.* **3**, 784.

ORGEL L.E. (1963) The maintenance of accuracy of protein synthesis and its relevance to aging. *Proc. nat. Acad. Sci.* **49**, 517.

PERKINS E.H. (1971) Phagocytic activity of aged mice. *J. Reticuloendothel. Soc.* **9**, 642.

PLAYFAIR J.H.L. (1968) Strain differences in the immune response of mice. I. The neonatal response to sheep red cells. *Immunology*, **15**, 35.

PRESS J.L. & KLINMAN N.R. (1973) Enumeration and analysis of antibody-forming cell precursors in the neonatal mouse. *J. Immunol.* **111**, 829.

PRICE G.B. & MAKINODAN T. (1972a) Immunologic deficiencies in senescence. I. Characterization of intrinsic deficiencies. *J. Immunol.* **108**, 403.

PRICE G.B. & MAKINODAN T. (1972b) Immunologic deficiencies in senescence. II. Characterization of extrinsic deficiencies. *J. Immunol.* **108**, 413.

RABINOWITZ S.G. (1976) Measurement and comparison of the proliferative and antibody responses of neonatal, immature and adult murine spleen cells to T-dependent and T-independent antigens. *Cell. Immunol.* **21**, 201.

RITTENBERG M.B. & PRATT K.L. (1969) Anti-trinitrophenyl (TNP) plaque assay. Primary response of Balb/c mice to soluble and particulate immunogen. *Proc. Soc. exp. Biol. Med.* **132**, 575.

ROSENBERG Y.J. & CUNNINGHAM A.J. (1975) Ontogeny of the antibody-forming cell line in mice. Kinetics of appearance of mature B cells. *Europ. J. Immunol.* **5**, 444.

SCHER I., SHARROW S.O., WISTER R., ASOFSKY R. & PAUL W.E. (1976) B-lymphocyte heterogeneity: ontogenic development and organ distribution of B-lymphocyte populations defined by their density surface immunoglobulin. *J. exp. Med.* **144**, 494.

SEGRE D. & SEGRE M.A. (1976a) Humoral immunity in aged mice. II. Increased suppressor T cell activity in immunologically deficient old mice. *J. Immunol.* **116**, 735.

SEGRE M.A. & SEGRE D. (1976b) Humoral immunity in aged mice. I. Age-related decline in the secondary response to DNP of spleen cells propagated in diffusion chambers. *J. Immunol.* **116**, 731.

SIDMAN C.L. & UNANUE E.R. (1975) Development of B lymphocytes. I. Cell populations and a critical event during ontogeny. *J. Immunol.* **114**, 1730.

SPEAR P.G. & EDELMAN G.E. (1974) Maturation of humoral immune response *in vitro*. *J. exp. Med.* **139**, 249.

STEEL R.G.D. & TORRE J.H. (1960) *Principles and Procedures of Statistics*. McGraw-Hill, Inc., London.

STREHELER B.L., HIRSCH G., GUSSECK D., JOHNSON R. & BICH M. (1971) Codon restriction theory of aging and development. *J. Theor. Biol.* **33**, 429.

TADA T., TANIGUCHI M. & TAKEMORI T. (1975) Properties of primed suppressor T cells and their products. *Transplant. Rev.* **26**, 106.

TAKEMORI T. & TADA T. (1974) Selective roles of thymus-derived lymphocytes in the antibody response. II. Preferential suppression of high-affinity antibody-forming cells by carrier-primed suppressor T cells. *J. exp. Med.* **140**, 253.

VITETTA E.S., MELCHERS U., MCWILLIAMS M., LAMM M.E., PHILLIPS-QUAGLIATA J.M. & UHR J.W. (1975) Cell surface immunoglobulin. IX. The appearance of an IgD-like molecule on murine lymphoid cells during ontogeny. *J. exp. Med.* **141**, 206.

VON HAHN H.P. (1973) Failures of regulation mechanisms as causes of cellular aging. *Advan. Gerontol.* **3**, 1.