Aging related changes of circadian rhythmicity of cytotoxic lymphocyte subpopulations

Gianluigi Mazzoccoli*, Angelo De Cata1, Antonio Greco1, Marcello Damato1, Nunzia Marzulli1, Mariangela Pia Dagostino1, Stefano Carughii1, Federico Perfetto2 and Roberto Tarquini2

Abstract

Background: Immunoseneescence is a process that affects all cell compartments of the immune system and the contribution of the immune system to healthy aging and longevity is still an open question. Lymphocyte subpopulations present different patterns of circadian variation and in the elderly alteration of circadian rhythmicity has been evidenced. The aim of our study was to analyze the dynamics of variation of specific cytotoxic lymphocyte subsets in old aged subjects.

Methods: Lymphocyte subpopulation analyses were performed and cortisol serum levels were measured on blood samples collected every four hours for 24 hours from fifteen healthy male young-middle aged subjects (age range 36-55 years) and fifteen healthy male old aged subjects (age range 67-79 years).

Results: In healthy young-middle aged subjects CD20 were higher and at 06:00 h CD8+ dim correlated positively with CD16+ and positively with γδTCR+ cells, CD16 correlated positively with γδTCR+ cells At 18:00 h CD8+ dim correlated positively with CD16+ and positively with γδTCR+ cells, CD16+ correlated positively with γδTCR+ cells and a clear circadian rhythm was validated for the time-qualified changes of CD3+, CD4+, CD20+, CD25+ and HLA-DR+ cells with acrophase during the night and for the time-qualified changes of CD8+, CD8+ bright, CD8+ dim, CD16+ and γδTCR+ cells with acrophase during the day. In old aged subjects CD25, DR+ T cells and cortisol serum levels were higher, but there was no statistically significant correlation among lymphocyte subpopulations and a clear circadian rhythm was evidenced for time-qualified changes of CD3+ and CD25+ cells with acrophase during the night and for the time-qualified changes of CD8+ cells and cortisol with acrophase during the day.

Conclusion: Our study has evidenced aging-related changes of correlation and circadian rhythmicity of variation of cytotoxic lymphocyte subpopulations that might play a role in the alteration of immune system function in the elderly.

Background

There are a number of reports in the scientific literature that put in evidence a circadian rhythm of variation of total lymphocytes in the peripheral blood, of antibodies and cell mediated immune responses [1,2] and an inverse relationship with plasma cortisol concentration [3]. Alteration of circadian rhythmicity has been evidenced in the elderly. A small fraction of peripheral T cells coexpress CD4 and low levels of CD8 (CD4+CD8dim) and can have cytotoxic activity. NK receptors are constitutively expressed and inducible on CD8+ cells upon antigen exposure or the cellular stress and cell-mediated cytotoxicity functions through non-major histocompatibility complex (MHC) or MHC-restricted mechanisms. MHC-restricted cytotoxicity is mainly mediated by CD8+ cytotoxic T lymphocytes through two distinct perforin- and Fas-based pathways resulting in tissue destruction [4], γδ-TCR expressing T cells represent a distinct mature T-cell lineage with the capacity to proliferate in response to receptor-mediated signals and to display non-MHC-restricted cytolsysis [5,6]. Natural killer (NK) cells are large granular lymphocytes that express neither αβ or γ/δ TCR nor CD3 on their surface and can lyse a number of different tumour cells. NK cells originate from bone marrow, but can mature in a variety of primary and secondary lymphoid tissues and the interaction with dendritic cells seems to be required for their optimal activation.

* Correspondence: g.mazzoccoli@tin.it
1 Department of Internal Medicine, Scientific Institute and Regional General Hospital "Casa Sollievo della Sofferenza", S.Giovanni Rotondo (FG), Italy
Full list of author information is available at the end of the article

© 2010 Mazzoccoli et al; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
The two key effector functions of human NK cells are killing and cytokine production and NK cells could mediate tissue damage and regulate autoimmune T-cell responses through cytokine secretion and cytotoxicity in secondary lymphoid organs [7].

Cytotoxic T lymphocytes are part of the adaptive immune system, natural killer cells are part of the innate immune system, and γδ-TCR expressing T cells may represent a functional and/or temporal bridge between this two cellular arms and may link the two major functional modality of immune response. These three cellular subsets differ in killing repertoire, but their function is of utmost importance for the body defence against foreign cells, cancer cells and cells infected with a virus.

In this study we investigated physiological variations of specific cytotoxic T lymphocyte subsets in old aged subjects.

**Methods**

Subjects gave written informed consent and the study was approved by the local Scientific and Ethical Committee. Peripheral blood samples were collected at intervals of four hours for twenty four hours from fifteen healthy male young and middle aged subjects (range 36-55 years, mean age ± s.e. 44.1 ± 1.7) and fifteen healthy male old aged subjects (range 67-79 years, mean age ± s.e. 68.5 ± 1.2). Inclusion criteria were age (< 65 years for young and middle aged subjects and ≥ 65 and < 80 years for old aged subjects), BMI (> 25 and < 30), no smoking status, normal physical activity level, no psychiatric disorder, no alcohol intake, no chronic conditions, and normal blood pressure level. In all subjects healthy status was assessed by medical history and physical examination, basal screening and after centrifugation the supernatants were washed with PBS. Non-lymphocytic cells contaminating the preparations were excluded from analysis using scatter gates set on the 90° light scatter profile. At least 10000 cells were acquired on the FACScan. Absolute counts of T cell subsets were calculated based on the proportion of the respective T cell subpopulation and on absolute counts obtained by the procedure. The number of fluorescent cells was expressed as a percentage of the total lymphocytes. To measure hormone serum concentrations blood samples were centrifuged immediately after collection and frozen at -20°C for later determination. All samples were analyzed in duplicate in a single assay; the intrassay and interassay coefficients of variation were below respectively 10% and 9% using a polarized light immuno-fluorescence assay (Cortisol TDX/TDXFLx, Abbott Laboratories, Abbott Park, Illinois, USA).

**Statistical analysis**

Statistical evaluation of percentages of cells was performed by non-inferential descriptive biometric analysis (Pearson's product moment correlation coefficients and linear regression calculated for percentages of cells at each sampling time to assess temporal relationships between variations in lymphocyte subpopulations and Student's t test and Mann-Whitney rank sum test, as indicated, on areas under the curve, calculated according to the trapezoidal method; a p value ≤ 0.05 was considered significant) and by an inferential temporal descriptive biometric analysis using the methods named Single Cosinor and Population Mean Cosinor, based on a least square fit of a cosine wave to individual and group time series data, testing the occurrence (whether the zero-amplitude assumption is rejected at a probability level p ≤ 0.05) and quantifying the parameters MESOR, Amplitude and Acrophase of consistent pattern of circadian rhythm. MESOR is the acronym for Midline Estimating Statistic of Rhythm and defines the rhythm-determined average. Amplitude is the measure of one half the extent of rhythmic change in a cycle estimated by the function used to approximate the rhythm. Acrophase, measure of timing, is the phase angle of the crest time in the function appropriately approximating a rhythm, in relation to the specified reference timepoint. Rhythms with a frequency of 1 cycle per 20 ± 4 h are designated circadian, rhythms with a frequency higher than 1 cycle per 24 h are designated as ultradian, and rhythms with a frequency lower than 1 cycle per 24 h are designated as infradian [8].

Abbott Laboratories, Abbott Park, Illinois, USA).
Results
Table 1 shows the human clusters of differentiations (CDs). Table 2 shows integrated time-qualified 24-hours values expressed as area under the curve (AUC) ± SE, with a statistically significant difference for the AUC values of CD20 (higher in young-middle aged subjects, p < 0.01) and for the AUC values of CD25, DR+ T cells and cortisol (higher in old aged subjects, p < 0.01, p = 0.01 and p = 0.04 respectively). Table 3 shows chronobiologically data derived from best fitting sine curves (fitted period: 24 hours = 360°): in young middle aged subjects a clear circadian rhythm was validated for the time-qualified changes of CD3+, CD4+, CD20+, CD25+ and HLA-DR+ cells with acrophase during the night and for the time-qualified changes of CD8+, CD8+ bright, CD8+ dim, CD16+, γδTCR+ cells and cortisol with acrophase during the day. In old aged subjects a clear circadian rhythm was evidenced for the time-qualified changes of CD3+ and CD25+ cells with acrophase during the night and for the time-qualified changes of CD8+ cells with acrophase during the day. Figure 1 shows correlations among lymphocyte subpopulations in the photoperiod (06:00h-10:00h-14:00h): in young-middle aged subjects at 06:00 h CD8+ dim correlated positively with CD16+ (r = 0.803, p < 0.001) and positively with γδTCR+ cells (r = 0.603, p = 0.005), CD16 correlated positively with γδTCR+ cells (r = 1.138, p < 0.001), whereas in old aged subjects there was no statistically significant correlation among lymphocyte subpopulations. Figure 2 shows correlations among lymphocyte subpopulations in the scotoperiod (18:00h-22:00h-02:00h): in young-middle aged subjects at 18:00 h CD8+ dim correlated positively with CD16+ (r = 0.852, p < 0.001) and positively with γδTCR+ cells (r = 1.012, p = 0.05), CD16+ correlated positively with γδTCR+ cells (r = 1.676, p < 0.001), whereas in old aged subjects there was no statistically significant correlation among lymphocyte subpopulations. In young middle aged subjects cortisol correlated negatively with CD16 (r = -0.486, p = 0.04), with CD20 (r = -0.464, p < 0.001), with CD25 (r = -0.489, p = 0.04) and with γδTCR+ cells (r = -0.509, p = 0.02) at 06:00 h.

Figure 3 shows 24-hour time qualified profiles of lymphocyte subset percentages and cortisol serum levels expressed as mean ± SE calculated on single time point values.

Discussion
Cellular immune responses drive all adaptive immunity, lymphocyte subpopulations present circadian variation of some of their subsets and this variation may influence magnitude and expression of the immune responses [9-13]. Aging associated changes have been demonstrated not only in T lymphocytes but also in different aspects of the innate immunity including natural killer (NK) cells [14,15]. The CD8+ lymphocytes are heterogeneous in subphenotypes and functions and include T cells, which express high-density CD8 (CD4-CD8bright+) and T cells, which express low-density CD8 (CD4+CD8dim+). CD8+ T lymphocytes expressing CD8dim represent cytotoxic effector populations and contain high amounts of perforin, which explains their greater cytolytic capacity [16,17]. A distinct subset of CD3+CD4+CD8-T lymphocytes expresses a CD3-associated heterodimer made up of the protein encoded by the T-cell receptor (TCR) gamma-gene and a second glycoprotein termed TCR delta. TCR gamma-delta (γδ-TCR) is expressed on CD3+ thymocytes during fetal ontogeny before the appearance of TCR alpha-beta (γδ-TCR), on CD3+CD4-CD8-adult thymocytes, and on a subset (1-10%) of CD3+ cells in adult peripheral lymphoid organs and the peripheral blood. γδ-TCR expressing T cells probably represent a distinct mature T-cell lineage with the capacity to proliferate in response to receptor-mediated signals, and to display non-major histocompatibility complex (MHC)-restricted cytosis [18,19]. NK cells are large granular lymphocytes that express neither α/β or γ/δ TCR nor CD3 on their surface, can lyse a number of different

Table 1: Human Clusters of Differentiations (CDs)

| CD3   | the signaling component of the T cell receptor (TCR) complex, found on T cells |
| CD4   | a co-receptor for MHC Class II, found on T helper/inducer subset |
| CD8   | a co-receptor for MHC Class I, found on T suppressor/cytotoxic subset |
| CD16  | FcγRII, a low-affinity Fc receptor for IgG, found on NK cells, macrophages, and neutrophils |
| CD20  | a type III transmembrane protein found on B cells |
| CD25  | a type I transmembrane protein found on activated T cells that associates with CD122 to form a heterodimer that can act as a high-affinity receptor for IL-2 |
| HLA-DR| a transmembrane human major histocompatibility complex (MHC) II family member expressed primarily on B cells on which it presents antigenic peptides for recognition by the T cell receptor on CD4+ T cells. |
| TcRδ1 | epitope of the constant domain δ of chain of TCR found on γδTCR expressing cells |
tumour cells and may be stimulated by IFN-γ, IL2, IL12 and IL18. The molecular and structural properties of γδ-TCR, the physiological role of T lymphocytes expressing γδ-TCR and the relationship between CD3+αβ T lymphocytes, NK and CD3+γδ T lymphocytes are still a matter of investigation.

There are different circadian variations of the total number of circulating immune cells and of specific lymphocyte subpopulations and the different compartmentalization of lymphocytes in space and in time has major functional consequences and leads to a partial fragmentation of immunoregulatory circuits at the local level [20-25]. The total number of circulating lymphocytes changes with circadian rhythmicity in antiphase with cortisol [26-28] and in our study we have evidenced that this rhythm of variation is recognizable for the changes of CD3 (total T cells), CD4 (T helper/inducer subset), CD20 (total B cells), CD25 (activated T lymphocytes with expression of α chain of IL2 receptor), HLA-DR (B cells and activated T cells) higher during the night, whereas CD8, CD8 bright and CD8 dim (T suppressor and cytotoxic lymphocytes respectively), CD16 (natural killer) and TcRδ1 (γδTCR expressing cells) are higher around noon. These opposing circadian variations resemble a temporal organization of cellular immune function. Naive T lymphocytes need to be activated and subsequently differentiate into effector cells to perform their immune functions. Regulation of T-cell responses involves diverse strategies of activation and inhibition to optimize recognition of infected or transformed cells, while preventing tissue damage as a result of autoreactivity and chronic inflammation. TCR-CD3-dependent responses are regulated by constitutive or inducible expression of costimulatory and inhibitory receptors, such as CD28 and its CTLA-4 counterpart. In recent years, however, it has become evident that the expression of NK cell receptors of the NKG2 family (eg, NKG2D and CD94/NKG2 receptors) on CD8+αβ+ effector T cells may represent another mean to regulate cytolytic functions in the tissue microenvironment, effectively controlling antigen-specific killing. NKG2D is one of the most widely distributed "NK-cell receptors," being expressed at the surface of all CD8+αβ+ T cells, γδ T cells, NK cells, as well as on certain activated CD4+ T cells. NKG2D is a potent costimulator of TCR-mediated effector functions and up-regulates antigen-specific T cell-mediated cytotoxicity directed against cells or tissues expressing stress-induced NKG2D ligands (NKG2DLs), particularly under conditions of suboptimal TCR engagement [29-36]. As evidenced in our study in healthy young-middle aged subjects the CD8dim+ T cytotoxic lymphocytes, NK cells and the γδ-TCR expressing cells show evident positive statistical correlation and a clear circadian rhythmicity of variation with higher levels during the photoperiod (figure 1, 2 and 3) and as evidenced in the scientific literature they share costimulators and ligands, suggesting that cytotoxic cell compartment is tightly connected in time and maybe function. The surface molecules and the mechanisms involved in the activation of γδ-TCR+ cells are similar to those of αβ-TCR+ cells and activated γδ-TCR+ cells have strong cytotoxic effector activity using both death receptor/death ligand and cytolytic granule pathways and produce various cytokines, frequently including tumor necrosis factor-α and IFN-γ [37,38]. Most CD3+γδ expressing T cell lines mediate cytotoxicity against a broad spectrum of tumour-cell targets, although the functional significance of this observation remains unclear [39]. An hypothesis is that γδ-TCR expressing cells recognize subtle alterations in

---

Table 2: Integrated time-qualified 24-hours values expressed as AUC ± SE

|                     | Healthy young-middle aged subjects | Healthy old aged subjects |
|---------------------|------------------------------------|---------------------------|
| CD3                 | 1545.41 ± 42.23                    | 1576.07 ± 25.85           |
| CD4                 | 891.33 ± 60.52                     | 837.41 ± 32.41            |
| CD8                 | 603.73 ± 92.12                     | 615.24 ± 30.21            |
| CD4/CD8 ratio       | 39.40 ± 12.71                      | 31.53 ± 1.35              |
| CD16                | 142.50 ± 45.30                     | 171.72 ± 31.63            |
| CD20                | 264.12 ± 30.84                     | 132.78 ± 21.23            |
| CD25                | 76.12 ± 14.21                      | 140.02 ± 24.25            |
| DR+T cells          | 61.8 ± 10.23                       | 109.5 ± 8.31              |
| HLA-DR              | 327.05 ± 23.40                     | 282.57 ± 20.42            |
| TcRδ1               | 61.72 ± 13.71                      | 86.23 ± 9.25              |
| Cortisol            | 258.2 ± 13.4                       | 310.6 ± 32.7              |

Units: % for lymphocyte subpopulations, μg/dl for cortisol all; parameters analyzed in all the subjects; p < 0.05•
host cells that may be associated with neoplastic transformation. In our old aged subjects we have evidenced decrease of B cell compartment, that may cause diminished response to immunological stimulation, increase of activated T cell compartment, that may be responsible of increased autoimmunity phenomena and a severe alteration of circadian rhythmicity of variation of natural killer and γδ-TCR bearing cells with loss of physiological timed windows of interaction. This phenomenon may be very important and dangerous, considered that these cells might represent the true immune surveillance cells [40] and may contribute to the increased incidence of cancer in old aged people, working with the accumulating DNA damage produced by chemicals, physical agents, free radicals and a number of carcinogens widely contaminating the environment of our daily living.

In our old aged subjects we have evidenced higher cortisol serum levels with circadian rhythmicity of secretion characterized by advance in acrophase. These data are in agreement with those reported in the international litera-

### Table 3: Chronobiological data derived from best fitting sine curves (fitted period: 24 hours = 360°)

| Factor | p   | MESOR ± SE | Amplitude ± SE | Acrophase ± SE(°) | Time (Hh:Mn) |
|--------|-----|------------|----------------|-------------------|--------------|
| CD3    | 0.002 | 78.06 ± 0.10 | 1.12 ± 0.22 | 25.1 ± 12.4 | 01:40 ± 00:50 |
| CD4    | 0.001 | 45.23 ± 0.85 | 3.14 ± 1.12 | 3.3 ± 24.5 | 00:13 ± 01:38 |
| CD8    | 0.003 | 29.52 ± 0.23 | 1.94 ± 0.25 | 181.3 ± 2.4 | 12:05 ± 00:10 |
| CD8 bright | 0.001 | 21.43 ± 0.11 | 1.49 ± 0.21 | 187.3 ± 10.2 | 12:29 ± 00:41 |
| CD8 dim | 0.002 | 8.09 ± 0.14 | 1.33 ± 0.09 | 192.1 ± 3.8 | 12:48 ± 00:15 |
| CD4/CD8 ratio | 0.001 | 1.53 ± 0.02 | 0.23 ± 0.1 | 16.0 ± 0.2 | 01:04 ± 00:01 |
| CD16   | 0.030 | 6.26 ± 0.42 | 0.81 ± 0.21 | 212.1 ± 21.3 | 14:08 ± 01:25 |
| CD20   | 0.002 | 13.23 ± 0.24 | 1.51 ± 0.11 | 336.8 ± 12.2 | 22:27 ± 00:49 |
| CD25   | 0.002 | 3.82 ± 0.02 | 0.67 ± 0.21 | 9.2 ± 7.1 | 00:37 ± 00:28 |
| DR+T cells | 0.005 | 3.21 ± 0.30 | 0.83 ± 0.20 | 12.2 ± 51.2 | 00:49 ± 03:25 |
| HLA-DR | 0.010 | 16.22 ± 0.25 | 1.33 ± 0.33 | 332.6 ± 11.3 | 22:10 ± 00:45 |
| TcRδ1  | 0.001 | 12.02 ± 0.09 | 0.63 ± 0.12 | 159.5 ± 14.3 | 10:38 ± 00:57 |
| Cortisol | 0.011 | 10.23 ± 1.42 | 6.51 ± 2.52 | 141.6 ± 22.1 | 09:26 ± 01:28 |
| Factor | p   | MESOR ± SE | Amplitude ± SE | Acrophase ± SE(°) | Time (Hh:Mn) |
|--------|-----|------------|----------------|-------------------|--------------|
| CD3    | 0.002 | 84.91 ± 0.21 | 1.07 ± 0.02 | 71.2 ± 3.0 | 04:45 ± 00:12 |
| CD4    | 0.145 | 45.12 ± 0.89 | 3.10 ± 1.27 | 33.2 ± 22.1 | 02:13 ± 01:28 |
| CD8    | 0.005 | 29.21 ± 0.23 | 3.25 ± 0.53 | 188.9 ± 11.2 | 12:36 ± 00:45 |
| CD8 bright | 0.001 | 20.12 ± 0.08 | 1.47 ± 0.19 | 196.1 ± 12.3 | 13:04 ± 00:49 |
| CD8 dim | 0.002 | 9.09 ± 0.15 | 1.31 ± 0.87 | 191.7 ± 2.4 | 12:47 ± 00:10 |
| CD4/CD8 ratio | 0.001 | 1.48 ± 0.06 | 0.22 ± 0.04 | 17.9 ± 11.7 | 01:12 ± 00:47 |
| CD16   | 0.246 | 8.02 ± 0.31 | 2.42 ± 0.43 | 191.3 ± 7.5 | 12:45 ± 00:30 |
| CD20   | 0.210 | 8.36 ± 0.16 | 1.15 ± 0.11 | 287.2 ± 21.4 | 19:09 ± 01:26 |
| CD25   | 0.031 | 7.12 ± 0.13 | 1.02 ± 0.21 | 251.2 ± 12.3 | 16:45 ± 00:49 |
| DR+T cells | 0.057 | 5.12 ± 0.35 | 1.73 ± 0.2 | 141 ± 11.2 | 09:24 ± 00:45 |
| HLA-DR | 0.297 | 13.21 ± 0.12 | 1.21 ± 0.9 | 185.1 ± 33.2 | 12:20 ± 02:13 |
| TcRδ1  | 0.210 | 4.28 ± 0.12 | 0.32 ± 0.11 | 192.1 ± 30.4 | 12:48 ± 02:02 |
| Cortisol | 0.017 | 13.26 ± 0.40 | 5.42 ± 1.31 | 121.8 ± 10.2 | 08:07 ± 00:40 |

Units: % for lymphocyte subpopulations, g/dl for cortisol, all parameters analyzed in all the subjects
Figure 1 Correlations between lymphocyte subpopulations in the photoperiod. In the photoperiod (06:00h-10:00h-14:00h) CD8+ dim correlates positively with CD16+ ($r = 0.803, p < 0.001$) and positively with γδTCR+ cells ($r = 0.603, p = 0.005$). CD16 correlates positively with γδTCR+ cells ($r = 1.138, p < 0.001$). There is no statistically significant correlation in old aged subjects.
Figure 2 Correlations between lymphocyte subpopulations in the scotoperiod. In the scotoperiod (18:00h-22:00h-02:00h) CD8+ dim correlates positively with CD16+ ($r = 0.852, p < 0.001$) and positively with γδTCR+ cells ($r = 1.012, p = 0.05$), CD16+ correlates positively with γδTCR+ cells ($r = 1.676, p < 0.001$). There is no statistically significant correlation in old aged subjects.
ture describing that the circadian profile of plasma cortisol is conserved in the elderly, but with higher plasma levels during the night [41]. The human adrenals show a marked circadian periodicity in the response to endogenous ACTH, with an acrophase in the morning in young adult subjects and with relative resistance to endogenous ACTH stimulation in the evening hours. In the elderly this rhythm shows a marked decrease in amplitude, with similar response to ACTH during daytime and evening hours and this phenomenon causes an elevated cortisol 24 h mean level and a reduction in the rhythm amplitude[42]. The higher plasma cortisol levels at night may play a role in the cognitive and metabolic disturbances found in the elderly and in the immune changes found in our old aged subjects. Cortisol circadian rhythm is a robust rhythm that does not respond rapidly to minor and transient environmental changes, which makes it a good candidate as a rhythm marker, but a trend for a phase advance in plasma cortisol has been reported in the elderly [43]. The higher plasma cortisol levels at night may play a role in the cognitive and metabolic disturbances found in the elderly and in the immune changes found in our old aged subjects. Cortisol circadian rhythm is a robust rhythm that does not respond rapidly to minor and transient environmental changes, which makes it a good candidate as a rhythm marker, but a trend for a phase advance in plasma cortisol has been reported in the elderly [43]. The immune system function is characterized by a complex time structure composed of multiple rhythms in different frequency ranges. The rhythms of the same frequency may have the same phase or different phases and usually show a well defined time-relation to each other. The loss of the array of rhythms or a change of their functional interactions may alter the organism’s time structure leading to chronodisruption and internal desynchronization. The alteration of the organism’s time structure may lead to functional disturbances and may impair repairing and defensive mechanisms. A complete loss of rhythmicity or a change of phase of the rhythms are the most frequent alterations, but another important factor is represented by the change of amplitude of variation. The multifrequency structure that characterizes the function of the immune system and the complexity of the time qualified variations of its different components has to be taken in consideration when we approach functional evaluations, clinical interpretations, and therapeutical interventions.

**Conclusion**

The aging immune cellular system is characterized by a severe alteration of circadian rhythmicity of the cytotoxic compartment that may be responsible for functional derangement with increased susceptibility to and reduced defense against neoplastic disease.

**Competing interests**

The authors declare that they have no competing interests.
the publication of this manuscript, either now or in the future. No organization is financing this manuscript (including the article-processing charge). We do not hold any stocks or shares in an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future.

We do not hold or are currently applying for any patents relating to the content of the manuscript. We have not received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript.

We have no other financial competing interests. There are no non-financial competing interests (political, personal, religious, ideological, academic, intellectual, commercial or any other) to declare in relation to this manuscript.

Authors’ contributions

GA: conception and design of the study, data collection, analysis and interpretation of data, carried out statistical analysis and the draft of the manuscript. AD: interpretation of data, carried out part of the draft of the manuscript. AG: interpretation of data, carried out part of the draft of the manuscript. MD: data collection, data interpretation, carried out part of the draft of the manuscript. NM: data collection, data interpretation, MPD: data collection, data interpretation, carried out part of the draft of the manuscript. SC: data collection, data interpretation, FP: data interpretation, carried out part of the draft of the manuscript. RT: critical review of the manuscript, interpretation of data. All the Authors have read and approved the submission of the present version of the manuscript and that the manuscript has not published and is not being considered for publication elsewhere in whole or in part in any language except as an abstract.

Author Details

1Department of Internal Medicine, Scientific Institute and Regional General Hospital “Casa Sollievo della Sofferenza”, S.Giovanni Rotondo (FG), Italy and 2Department of Internal Medicine, University of Florence, Florence, Italy

Received: 7 March 2010 Accepted: 25 May 2010
Published: 25 May 2010

References

1. Abo T, Kumagai K: Studies of surface immunoglobulins on human B lymphocytes. III. Physiological variations of SIg+ cells in peripheral blood. Clin Exp Immunol 1978, 33:441-452.

2. Kawate T, Abo T, Hinuma S, Kumagai K: Studies of the bioperiodicity of the immune response: II. Co-variations of murine T and B cells and a role of corticosteroid. J Immunol 1981, 126:136-1367.

3. Palm S, Peste E, Hinrichsen H, Maier H, Zabel P, Kirch W: Twenty-four-hour analysis of lymphocyte subpopulations and cytokines in healthy subjects. Chronobiol Int 1996, 13:423-434.

4. Lambert C, Ibrahim M, Iobagiu C, Genin C: Significance of unconventional peripheral CD4+CD8dim T cell subsets. J Clin Immunol 2005, 25:418-427.

5. Matis LA, Cron R, Bluemte JA: Major histocompatibility complex-linked specificity of gamma delta receptor-bearing T lymphocytes. Nature 1987, 330:262-264.

6. Moretta L, Ciccone E, Mingari MC, Bottino C, Ferrini S, Tambussi G, Melioli G, Grossi CE, Moretta A: Human T lymphocytes expressing gamma/delta T cell antigen receptor. Clin Immunol Immunopathol 1989, 50:117-123.

7. Arjona A, Boyadjieva N, Sarkar DK: Specificity of delta gamma receptor bearing T cells. Semin Immunol 1991, 3:95-100.

8. Grossi CE, Ciccone E, Zeromski J, Moretta A, Moretta L: Functional and morphologic characterization of human T lymphocytes expressing the TCR gamma/delta. Biotherapy 1992, 5:1-9.

9. van Andrian UH, Mempel TR: Homing and cellular traffic in lymph nodes. Nat Rev Immunol 2003, 3:867-878.

10. Matsuoka E, Aoki C, Sasaoka K, Hata A: Analysis of T-cell subpopulations and cytokines in peripheral blood of healthy middle-aged men and women. Chronobiol Int 1999, 16:581-622.

11. Haus E, Smolenisky MH: Biologic rhythms in the immune system. Chronobiol Int 1999, 16:581-622.

12. Fauci AS: Mechanism of corticosteroid function on lymphocyte subpopulations. I. Redistribution of circulating T and B lymphocytes to the bone marrow. Immunology 1975, 28:659-680.

13. Dimitrov S, Benedict C, Heutling D, Westermann J, Born J, Lange T: Cortisol and epithepinephrine control opposing circadian rhythms in T cell subsets. Blood 2009, 113:5134-5143.

14. Borrego F, Alonso MC, Galliano MD, Carracedo J, Ramirez R, Ostos B, Peña J, Solana R: NK phenotype markers and IL2 response in NK cells from elderly people. Exp Gerontol 1999, 34:253-265.

15. Solana R, Mariani E, Solana NK and NK/T cells in human senescence. Vaccine 2000, 18:1613-1620.

16. Prince H, Golding J, York: Characterization of Circulating CD4+ CD8+ Lymphocytes in Healthy Individuals Prompted by Identification of a Blood Donor with a Markedly Elevated Level of CD4+ CD8+ Lymphocytes. Clin Diagn Lab Immunol 1994, 5:597-605.

17. Wang B, Maje R, Greenwood R, Collins EJ, Frelinger JA: Naive CD8+ T Cells Do Not Require Costimulaton for Proliferation and Differentiation into Cytotoxic Effector. J Immunol 2000, 164:1216-1222.

18. Matsu LA, Bluestone JA: Specificity of gamma delta receptor bearing T cells. Semin Immunol 1991, 3:75-80.

19. Fudenberg HH: Cytokines. J Clin Immunol 1990, 10:85-97.

20. von Andrian UH, Mempel TR: Homing and cellular traffic in lymph nodes. Nat Rev Immunol 2003, 2:867-878.

21. Moser B, Loetscher P: Lymphocyte traffic control by chemokines. Nature Immunol 2000, 1:123-128.

22. Sallusto F, Geginat J, Lanzavecchia A: Central memory and effector memory T cell subsets: function, generation, and maintenance. Annu Rev Immunol 2004, 22:745-763.

23. Barter FC, Della CS, Halberg F: A map of blood and urinary changes related to circadian variations in adrenergic cortical function in normal subjects. Ann NY Acad Sci 1962, 98:869-983.

24. Calderon RA, Thomas DB: In vivo cyclic change in B-lymphocyte susceptibility to T-cell control. Nature (Lond) 1980, 285:662-664.

25. Ritchie AWS, Oswald I, Mcklem HS, Boyd JE, Elton RA, Jazwiska E, James K: Circadian variation of lymphocyte subpopulations: a study with monoclonal antibodies. Br Med J 1986, 286:1773-1775.

26. Miller AH, Spencer RL, Hassett J: Effects of selective type I and II adrenergic steroid agonists on immune cell distribution. Endocrinology 1994, 135:1934-1944.

27. Mazzone S, Gennarelli M, Franco G, Scara R, Porta R, Esposito V: Cytokines and lymphocyte function in elderly people. A review. Aging Cell 2001, 3:101-105.

28. Cerboni C, Ardolino M, Santoni A, Zingoni A, Tarquini A: Age-related changes of neuro-endocrine-immune interactions in healthy humans. J Biol Regul Homeost Agents 1997, 11:143-147.

29. Chiappelli F, Gormley GJ, Wirstem EH: Effects of intravenous and oral dexamethasone on selected lymphocyte subpopulations in normal subjects. Psychoneuroendocrinology 1992, 17:145-152.

30. Cerboni C, Ardolino M, Santoni A, Zingoni A: Detuning CD8+ T lymphocytes by down-regulation of the activating receptor NKGD2: role of NKGD2 ligands released by activated T cells. Blood 2009, 113:2955-2964.

31. Marcelli C, Scaramuzza S, Perriani G: TNK cells (NKGD2+ CD8+ or CD4+ T lymphocytes) in the control of human tumors. Cancer Immunol Immunother 2009, 58:801-808.

32. Alonso-Arias R, Lopez-Vazquez A, Diaz-Pern S, Sampere AL, Casas L, Asensi V, Rodrigo L, Lopez-Larrea C: Specificity of gamma delta receptor-bearing T lymphocytes. Clin Exp Immunol 1994, 98:697-605.

33. Wang B, Maje R, Greenwood R, Collins EJ, Frelinger JA: Naive CD8+ T Cells Do Not Require Costimulaton for Proliferation and Differentiation into Cytotoxic Effector. J Immunol 2000, 164:1216-1222.

34. Moser B, Loetscher P: Lymphocyte traffic control by chemokines. Nature Immunol 2000, 1:123-128.

35. Sallusto F, Geginat J, Lanzavecchia A: Central memory and effector memory T cell subsets: function, generation, and maintenance. Annu Rev Immunol 2004, 22:745-763.
35. Rincon-Orozco B, Kunzmann V, Wrobel P, Kabelitz D, Steinle A, Hermann T. Activation of V(9gama)9V(delta)2 T Cells by NKG2D. J Immunol 2005, 175:2144-2151.

36. Vivier E, Tomasello F, Paul P. Lymphocyte activation via NKG2D: towards a new paradigm in immune recognition? Curr Opin Immunol 2002, 14:306-311.

37. Parmiani G. Tumor-infiltrating T cells-Friend or foe of neoplastic cells? N Engl J Med 2005, 353:2640-2641.

38. Pages F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, Mlecnik B, Kirilovsky A, Nilsson M, Damotte D, Meatchi T, Brunet P, Cugnenc PH, Trajanoski Z, Fridman WH, Galon J. Effector Memory T Cells, Early Metastasis, and Survival in Colorectal Cancer. N Engl J Med 2005, 353:2654-2666.

39. Bagot M, Echchakir H, Mami-Chouaib F, Delfau-Larue MH, Charue D, Bernheim A, Chouaib S, Bourmeille L, Benoussan A. Isolation of Tumor-Specific Cytotoxic CD4+ and CD4+CD8dim+ T-Cell Clones Infiltrating a Cutaneous T-Cell Lymphoma. Blood 1998, 91:4331-4341.

40. Kabelitz D, Wesch D, He W. Perspectives of gammadelta T cells in tumor immunology. Cancer Res 2007, 67:5-8.

41. Touitou Y, Haus E. Aging of the human endocrine and neuroendocrine time structure. Ann N Y Acad Sci 1994, 719:378-397.

42. Touitou Y, Bogdan A, Haus E, Touitou C. Modifications of circadian and circannual rhythms with aging. Exp Gerontol 1997, 32:603-614.

43. Touitou Y, Haus E. Alterations with aging of the endocrine and neuroendocrine circadian system in humans. Chronobiol Int 2000, 17:369-390.

doi: 10.1186/1740-3391-8-6
Cite this article as: Mazzoccoli et al., Aging related changes of circadian rhythmicity of cytotoxic lymphocyte subpopulations Journal of Circadian Rhythms 2010, 8:6