Developmental and Comparative Immunology 82 (2018) 39–48

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

Diversity of aging of the immune system classified in the cotton rat (*Sigmodon hispidus*) model of human infectious diseases

Teun Guichelaar [a, *], Elisabeth A. van Erp [a, b], Jeroen Hoeboer [a], Noortje A.M. Smits [a], Cécile A.C.M. van Els [a], Daan K.J. Pieren [a], Willem Luytjes [a]

[a] Centre for Infectious Disease Control (Clb), National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands
[b] Laboratory of Pediatric Infectious Diseases, Department of Pediatrics, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands

ARTICLE INFO

Article history:
Received 1 November 2017
Received in revised form
28 December 2017
Accepted 29 December 2017
Available online 2 January 2018

Keywords:
Cotton rat
RSV
Biological aging
Lymphocytes

ABSTRACT

Susceptibility and declined resistance to human pathogens like respiratory syncytial virus (RSV) at old age is well represented in the cotton rat (*Sigmodon hispidus*). Despite providing a preferred model of human infectious diseases, little is known about aging of its adaptive immune system. We aimed to define aging-related changes of the immune system of this species. Concomitantly, we asked whether the rate of immunological alterations may be stratified by physiological aberrations encountered during aging.

With increasing age, cotton rats showed reduced frequencies of T cells, impaired induction of antibodies to RSV, higher incidence of aberrations of organs and signs of lipemia. Moreover, old animals expressed high biological heterogeneity, but the age-related reduction of T cell frequency was only observed in those specimens that displayed aberrant organs. Thus, cotton rats show age-related alterations of lymphocytes that can be classified by links with health status.

1. Introduction

Aging is accompanied by a plethora of physiological changes that increase the risk for morbidity in the elderly. Many studies on aging classify biological deviations that may develop primarily by chronological age. However, the nature of age-related conditions and the pace at which they develop vary between individuals (Belsky et al., 2017; Brodin et al., 2015; Jonker et al., 2013). This biological diversity between individuals complicates interpretation of data on health risks that may come with old age.

With respect to aging, deterioration of the immune system is of particular interest since it causes impaired protection to pathogens and lower responsiveness to vaccines in the elderly. Deteriorated immunity in humans is exemplified by influenza virus and respiratory syncytial virus (RSV) infections causing severe disease more frequently at old age (Jansen et al., 2007; Rappuoli et al., 2011), and by impaired capacity to produce antibodies and declining numbers of T cells (Fagnoni et al., 2000; McElhaney et al., 2016).

The elderly population shows high interindividual diversity in the degree of alterations of the immune system (Shen-Orr and Furman, 2013). However, a growing number of studies in humans, mice and rats now reveal previously unrecognized links between the state of the immune system and age-related pathologies (Furman et al., 2017; Gibson et al., 2009; Jonker et al., 2013; McElhaney et al., 2016; Ori et al., 2015). These findings suggest that age-related pathologies may be used to categorize the risk for disease because of impaired immunological protection at an individual level.

The cotton rat (*Sigmodon hispidus*) provides a useful model to study the relation between human infectious diseases and aging. Unlike mice and rats, cotton rats are highly susceptible to respiratory disease by infection with human influenza viruses and human RSV (Ottolini et al., 2005; Prince et al., 1978). They also show aging-related lower efficacy of immunity to these viruses (Boukhvalova et al., 2007; Curtis et al., 2002; Guichelaar et al., 2014). For decades this small animal species has therefore provided a preferred model for studying efficacy of human vaccine candidates and other experimental interventions in disease caused by human viruses (Boukhvalova et al., 2009). However, the immune system of this species is still rather unexplored. Tools that enable studying

* Corresponding author. Antonie van Leeuwenhoeklaan 9, 3720 BA, Bilthoven, The Netherlands.
E-mail address: teun.guichelaar@rivm.nl (T. Guichelaar).

https://doi.org/10.1016/j.dci.2017.12.026

immune cells, especially at the B and T cell level, of this species are scarce and need to be developed in order to study aging of the cotton rat immune system in more detail.

After the age of 65 years people become increasingly prone to disease by pathogens like influenza virus and respiratory syncytial virus, indicating a reduced capacity of the immune system to control infections in elderly over the age of 65 years (Jansen et al., 2007). Similar to this susceptibility in elderly humans, from the age of eight months and older cotton rats show significantly increased susceptibility to infection and reduced immunity (Boukhvalova et al., 2007; Curtis et al., 2002; Guichelaar et al., 2014). This indicates that eight months in cotton rats could be equivalent to 65 years in humans. The average life expectancy of colony-bred *Sigmodon hispidus* has been reported approximately sixteen months by some labs (Boukhvalova et al., 2007), whereas cotton rats in the wild have been reported to achieve a life span of six months (Niewiesk and Prince, 2002).

Here, we aimed to explore how basic parameters of the adaptive immune system alter with age in the cotton rat, and whether these immunological alterations may be linked to aging-related physiological anomalies in this species. We measured the level of antibodies induced by RSV and the composition of B- and T-cell populations at different ages and noticed aberrations of organs and changes in serum levels of urea, creatinine and glucose. In addition to aging of adaptive arms of the immune system, we examined aging of cotton rat innate immunity. To this end, we measured responsiveness to Toll-like receptor agonists of cells from cotton rats at young and old age. We observed a higher interindividual variation in the level of several of these parameters at old age than at young age. Among the variation at old age, we found that reduced frequencies of T cells are confined to those animals that show aberrations of their organs. Using novel tools to assess lymphocytes, the findings in our study illustrate that the rate of age-related changes of the immune system in the cotton rat may be classified by non-immune physiological parameters of aging rather than by chronological age.

2. Materials and methods

2.1. Inoculation of cotton rats

Adult cotton rats (*Sigmodon hispidus*) were inoculated with RSV, or with PBS as uninfected control, at the age of three months or eight months (Boukhvalova et al., 2007; Curtis et al., 2002; Guichelaar et al., 2014). They were obtained from a specific-pathogen-free breeding colony that has been kept at the animal facilities of the Institute for Translational Vaccination (Bilthoven, The Netherlands) for more than ten years. This in house colony has originated from a specific-pathogen-free breeding colony of Charles River Laboratories, The Netherlands. Inoculation was done intranasally (i.n.) with 10 μl containing 1×10^4 50% tissue culture infective dose ($TCID_{50}$) of recombinant human RSV as described previously (Guichelaar et al., 2014). Animal studies were approved by the Ethical Committee of RIVM. Animal handling was carried out in accordance with Dutch national legislation.

2.2. Preparation of respiratory syncytial virus

The virus used for inoculation of cotton rats was produced and purified as described in detail previously (Guichelaar et al., 2014). In brief, this recombinant human RSV was derived from RSV-X (strain 98-25147-X), a clinically derived serogroup A strain. Recombinant RSV was recovered from a plasmid encoding cDNA containing directed mutations in the intergenic regions of the RSV-X genome, as described previously. This resulted in infectious recombinant RSV particles that express all the viral proteins but express a slightly attenuated phenotype.

2.3. Section, collection of organs and preparation of single-cell suspensions

At one, two or three months after inoculation cotton rats were sacrificed under anesthesia by Ketamine plus Xylazine and bled via punction of the orbital plexus. Inspection of animals and organs for the occurrence of macroscopical aberrations was done by well-trained bio-technicians of the animal facilities of the Institute for Translational Vaccination (Bilthoven, The Netherlands). Prevalent aberrations noted were: swelling, redness and purulence of uterus or seminal vesicles, abnormal swelling of stomach, anemic liver, liver tumor, adhesion of lungs, swollen heart, swollen spleen, malformed/granular structures in kidney, hemorrhage-like appearance of lungs, cyst in thymus. Organs were scored by routine, either positive or negative (i.e. being aberrant or not, respectively), and scoring was not based on grades of pathology. Analyses of the occurrence of aberrations of organs was done in a double-blinded way, only after all other data had been collected and analyzed. Blood was collected in Vacuette Z Serum Sep Clot Activator tubes (Greiner Bio-One) to obtain serum at the times indicated. Spleen and lungs were kept cold on ice during transport and processing to single cell suspensions. Lungs were chopped and subsequently digested by Collagenase plus DNase-I (Roche, Sigma-Aldrich) at 37 °C for 30 min before processing lungs into single cell suspensions. Single-cell suspensions were prepared from spleen and lungs by meshing the organs through a 70 μm Cell Strainer (Corning, NY, USA). Erythrocytes were removed from single cell suspensions by lysis with ACK-lysisbuffer. White blood cells in single cell suspensions were counted with an automated cell counter (Casy Model TT, Innovatis, Penzberg, Germany). Samples containing 5×10^5 cells were processed for flow cytometry.

2.4. Analyses of innate immune responses

Single cell suspensions were prepared from spleens of cohorts of non-(RSV-) inoculated cotton rats and cultured for three days in the presence of 1 μg/mL of Toll-like receptor (TLR)4-agonist LPS (*Salmonella TV119*, Invivogen), 3 μg/mL of TLR7-agonist Gardiquimod (Invivogen), or 1 μM of the CpG oligonucleotide ODN1826 (Invivogen), or in medium without added TLR-agonists. Hereafter, cotton rat interleukin-6 was measured in culture supernatants by ELISA according to the manufacturer's protocol (R&D Systems).

2.5. Flow cytometry

Single cell suspensions were washed with PBS containing 2% fetal calf serum (FCS) prior to and after staining with commercially available antibodies. Cells were labeled with anti-cotton rat CD8-PE (R&D Systems, Abingdon, UK), LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Molecular Probes), and anti-mouse CD4-PerCP-Cy5.5 (clone GK1.5, Biolegend, San Diego, CA). Subsequent intracellular staining of T cells using FITC-conjugated anti-CD3zeta (Abcam, Cambridge, UK) and B cells using APC-conjugated anti-human CD79a (clone HM57, Abcam) (Pueschel et al., 2007) was done after fixation and permeabilisation using commercial buffers (eBioscience). Specificity of antibodies was confirmed by their matching isotype-controls. Cells were measured on a FACS Canto II (BD Biosciences) and data were analyzed with FlowJo single cell analysis software (FlowJo, LLC).