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Compromised IFN-gamma production in the elderly leads to both acute and latent viral antigen stimulation: contribution to the immune risk phenotype?

Abstract

The elderly suffer from an increased incidence of infectious disease, accompanied by increased morbidity and mortality. Interferon-g plays an important role in defense against intracellular pathogens such as mycobacteria and viruses. A reduced capacity to produce this cytokine in the elderly, as

demonstrated by our findings of significant decreases in IFN-g production in vitro on stimulation with bacterial products (LPS) or viral antigens (influenza vaccine), might therefore contribute to disease susceptibility. Moreover, accumulating data suggest that persistent infection with EBV and particularly CMV impacts upon the immune system in aging and may contribute to the Òimmune risk phenotypeÓ (IRP), which predicts remaining longevity in the very elderly. Using tetramer technology and IFN-g ELISPOT assays, we found that the commonly-observed clonal expansions of CD8+ T-cells in the elderly were for the most part poorly-functional CMV- and EBV-specific cells, expressing little CD28. The resulting accumulation of dysfunctional cells may lead to a reduction of the repertoire of functional T-cells available for responses to novel antigens. Further longitudinal studies are needed to demonstrate whether cytokines such as IFN-g are also part of the IRP. Improving the definition of the IRP will help to understand and thus prevent or reverse age-associated immune dysfunction.

Full text

Introduction

The elderly have an increased incidence of many infectious diseases, accompanied by increases in morbidity and mortality. This is considered to be associated with impaired immune responses due to age-associated decreased immune function [1]. Pulmonary infections, such as viral and bacterial pneumonia and influenza, all occur at higher rates in elderly people. It has been proposed that immunodeficiency in the elderly is primarily due to a dysfunction of T-cells. Most T-cells in young adults resemble naive cells, freshly emigrated from the thymus to the peripheral immune organs, but aging leads to a shift away from naive cells to a relative increase in antigen-experienced memory subsets. Although T-cells with the surface characteristics of memory cells clearly increase with age, it is still unclear whether the function of these cells is impaired by aging. Decreased immune function may be due to aging and/or disease, and both may be reflected by altered T-cell cytokine production. However, reports on age-related changes in the production of several cytokines typically produced by memory T-cells, such as interferon-g (IFN-g), are also often inconsistent, possibly for this reason.

It is now clear that cytokines regulate both the initiation and maintenance of the immune response and serve as a measure of the functional immune status.

Particularly, IFN-g plays an important role in the defense against intracellular pathogens requiring cell-mediated immunity (e.g. mycobacteria and viruses). The IFN-g pathway has been implicated in the immune response to many infectious agents, including fungi, parasites and bacteria. The ability to inhibit virus replication is a first line host defense in the control of viral infections. Therefore, it is important to investigate the status of IFN-g production in the elderly in comparison with healthy young individuals and to try to establish the contribution of the state of health to the results obtained. Moreover, a comparison of responses to infection with an acute agent (influenza) and a chronic agent (CMV) might yield important information on the state of the immune system in the elderly. To this end, we first investigated IFN-g production in the elderly after stimulation with bacterial products (lipopolysaccharide; LPS) or viral antigen (influenza vaccine).

Interferon-g production in the elderly

Whole blood assays and PBMC assays were employed for comparing IFN-g production in frail but healthy elderly with healthy young persons after stimulation with LPS or influenza vaccine at different concentrations *ex vivo*. This study revealed that in the healthy young and healthy but frail elderly, decreased IFN-g production is associated with increased age, *regardless of health status* ($p < 0.001$). On the other hand, TNF-a production in the healthy elderly was only slightly lower as compared to the young ($p = 0.06$), whereas IL-10 production did not differ between healthy young and elderly subjects ($p = 0.73$).

Importantly, using the same assay, we observed a significantly decreased IFN-g production after stimulation with influenza vaccine in the frail elderly as compared to healthy young ($p < 0.001$). Similarly, IFN-g production upon stimulation with influenza vaccine, as measured by a PBMC rather than whole blood assay, was 6 times higher in healthy young than in the healthy elderly ($p < 0.001$). Therefore, we concluded that significant decreases in the IFN-g responses to bacterial products (LPS) and virus antigen (influenza vaccine) were characteristic of the elderly, regardless of their health status [2].

Interferon-g production in aged people in response to viral activation

Influenza virus causes only localized infections of limited duration without persistence and causes productive infections limited largely to the superficial epithelial cells of the respiratory tract. It is thought to be completely eliminated from normal, previously unexposed mice after 10-12 days, and there is no evidence for the persistence of viral genome following respiratory challenge [3]. Like influenza, most viral infections in humans are successfully cleared by the immune system. However, one exception to this is the important family of viruses, the Herpes viruses. Thus, *Varicella zoster* virus (VZV), *Cytomegalovirus* (CMV), and Epstein-Barr virus (EBV), can establish chronic infections that are never fully cleared by immune mechanisms but are clearly maintained under immune control, as exemplified by reactivation under immunosuppression or stress in the elderly [4]. The persistence of several Herpes viruses in aged people and the IFN-g production or the frequency of IFN-g producing T-cells after stimulation with viral antigen peptides have been studied.

Varicella zoster virus (VZV) induces neuronal destruction and inflammation, and causes the principal problems of pain, interference with activities of daily living, and reduced quality of life in the elderly. It has the ability to establish a latent infection for the lifetime of the host and it retains the capacity after many years to emerge at unpredictable times to cause Herpes zoster (HZ) by VZV reactivation (shingles). Extrapolating from HZ epidemiological studies, it is calculated that the lifetime incidence rate of HZ is 10-20 % in the general population and as high as 50 % of a cohort surviving to age 85 years [5]. This event is more likely to occur in the elderly, partly because of an age-related decline in specific, cell-mediated immune responses to VZV. It has been reported by Zhang *et al.* that when human blood lymphocytes were stimulated with VZV antigen in limiting dilution cultures, and the amount of IFN-g measured in the supernatants, the frequency of wells containing IFN-g was lower in subjects < 19 or > 55 years of age, and the amount of IFN-g in positive wells was significantly lower in cultures of the older subjects' lymphocytes [6]. Thus, the age-related decline in the Th1 pathway (characterized by the ability to secrete IFN-g) may also account for the increase in *Varicella zoster* reactivation in elderly people.

The "immunological risk phenotype" (IRP)

Apart from VZV, accumulating data have suggested that CMV and EBV are also important for inducing persistent, perhaps permanent, changes in immune

biomarkers in aging, especially CMV [7]. Longitudinal studies identify a cluster of immune parameters including high CD8 and low CD4 percentages and poor T-cell proliferation, which is associated with a higher 2-year mortality; seropositivity for CMV in the very old is part of this pattern [8]. This has lead to the development of the concept of the "immunological risk phenotype" (IRP) [9]. We studied whether there is a correlation between IRP and T-cell responses against viral antigens in the very elderly in terms of the frequency of viral antigen-specific T-cells and viral antigen-specific IFN-g production.

A very old population of > 87 years of age was studied by a combination of tetramer technology and ELISPOT assays. MHC/peptide tetramers for different CMV or EBV epitopes allow direct quantification of virus-specific T-cells on a single cell level. ELISPOT analysis allows the detection of virus-epitope-specific functional T-cells by identifying lymphocytes capable of secreting cytokines such as IFN-g in response to antigen stimulation.

The number of CMV antigen-specific $CD8^+$ T-cells was analyzed by staining PBMC with CMV tetrameric complexes (CMV epitope peptide NLVPMVATV derived from low matrix protein pp65, residues 495-503). The CMV antigen-specific IFN-g-producing T-cells were measured by ELISPOT after short-term stimulation with the same CMV epitope peptide. To assess the functional capacity of CMV antigen-specific T-cells, the proportion of IFN-g-producing T-cells *versus* tetramer-positive T-cells was evaluated in very old and young individuals. We found that the frequency of CMV-tetramer-positive cells within the $CD8^+$ subset was significantly greater in the very old compared to the young, especially in the IRP group (CD4/CD8 ratio less than one, $p < 0.001$). In marked contrast however, the proportion of these CMV antigen-specific T-cells actually able to produce IFN-g when stimulated with the appropriate antigen was significantly lower in the old. Sorting of tetramer-positive T-cells and subsequent ELISPOT analysis revealed that these findings were due to an accumulation of CMV-specific T-cells in the very elderly, which were not capable of producing IFN-g upon *in vitro* stimulation.

Similarly, an increase in EBV-specific cells is also seen in the very old, but not to such a striking degree as in CMV. The fraction of EBV epitope peptide GLCTLVAML (derived from the lytic cycle protein BMLF1, residues 280-288)-reactive T-cells producing IFN-g was significantly lower in the very old than in the young ($p < 0.001$).

Clonal expansions of virus-specific T-cells may therefore constitute an important part of the IRP. But because tetramer-positive T-cells have so far been sorted only from 2 individuals in the IRP group and from 3 individuals in the non-IRP group for determining IFN-g production, probably due to the small size of our study, we have not yet been able to find a direct correlation between IRP and the frequency of virus-specific IFN-g-producing T-cells in the very old.

Outlook

Taken together, these data confirm that CD8 clonal expansions occur in the elderly, and indicate that this results in an accumulation of dysfunctional cells carrying specific antigen receptors for very non-diverse viral epitopes. We hypothesize that, in the elderly, the "immunological space" is taken up by such dysfunctional T-cells, which results in the reduction of the available repertoire of T-cells for novel antigens. These findings may contribute to explain the increased incidence and case-fatality caused by viruses and intracellular pathogens in the elderly.

It is still not known which factors are responsible for determining whether elderly individuals will fall into the IRP category or not. Probably the interactions between genetic and environmental control of parameters such as cytokine levels are involved. It has been reported in a Danish, aging study that TNF-a was associated with mortality among men, whereas IL-6 was associated with mortality in both sexes [10]. The IRP may be related to cytokines involved in inflammatory processes. Further longitudinal studies are needed to demonstrate whether cytokines such as IFN-g are also part of the IRP, as implied by the data summarised in this short review. Improving the definition of IRP will help us to understand the basis for preventing or reversing age-associated immune dysfunction.

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