Disclosure of Financial Relationships

N.D. Vaziri M.D, MACP

NO, neither I nor my spouse/partner have anything to disclose.

Uremia and Immune Function

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Functions of the immune system

- The primary function of immune system is protection against disease by identifying and eliminating pathogens and tumor cells and by detecting, cleaning and facilitating repair of damaged tissues

- The central mechanism by which the immune system fulfils these tasks is "**inflammation**"

Innate and Adaptive Immune responses

- Innate immune system is responsible for mounting an immediate, generic and non-specific response to infection and tissue damage. Cells involved in Innate immunity include:

  Monocyte/macrophages, Neutrophils (PMN), Dendritic cells

  Mast cells, Eosinophils, Basophils, NK cells and nearly all other cells in the body

- Adaptive immune response provides the immune system with the ability to recognize and remember specific pathogens and to mount stronger attacks each time the pathogen is encountered Cells involved in Adaptive immunity include:

  T lymphocytes & B lymphocytes

ESRD is simultaneously associated with immune activation which is marked by systemic inflammation and immune deficiency which is evidenced by increased incidence, severity and mortality from microbial infections as well as impaired response to vaccination & anergy

Consequences of Uremia- induced immune disorder

- Systemic inflammation in ESRD contributes to atherosclerosis & cardiovascular disease (the most common cause of death), cachexia, anemia, among others

- Impaired immunity in ESRD contributes to diminished response to vaccination, increased incidence and severity of microbial infections (second most common cause of death in ESRD) & anergy

Objectives

This presentation is intended to review the functional and structural changes in immune cell populations that contribute to the pathogenesis of inflammation and impaired host response to microbial infections in ESRD

Cells orchestrating inflammation in CKD

CKD-associated inflammation & immune deficiency are, in part, due to activation & dysfunction of innate cells which include:

I- Innate immune cells

- **Monocytes/macrophages**
- **Polymorphonuclear leukocytes**

II- Non-immune cells

- **Essentially cells of all organs/tissues:**

(EC, VSMC, adipocytes, neuronal cells, renal cells, epithelial cells, others)

Pattern recognition molecules (PRM)

Innate immune and some non-immune cells express molecules that recognize pathogen-associated molecular patterns including:

- Secreted PRM (Mannose-binding lectin family)

  Elevated in ESRD

- Endocytic (SRA-1, CD36, LOX-1 etc.)

  Elevated in ESRD

- Signaling (Toll-like receptor family)

  Conflicting reports

Monocytes

Monocytes are produced by bone marrow, stored in spleen, and distributed in all body tissues as macrophages.

Functions

Monocytes/macrophages play a key role in a- **host defense** against bacterial infections, b-tissue **healing** process, and c- **inflammation** and **atherosclerosis** via:

- **Phagocytosis** of microbes, infected cells & tissue debris directly or via intermediary proteins (antibodies, complement)

- **Uptake of ox-LDL and ox-phospholipids** via scavenger receptors

- **Production of cytokines/growth factor, ROS, tissue factor, metalloproteinases**

Monocyte subtypes

- Monocytes are classified into 4 major subsets based on expressions of CD14 (pattern-recognition receptor) and CD16 (Fc gamma III receptor) :
  CD14++/CD16-; CD14++/CD16+;
  CD14+/CD16-; CD14+/CD16+

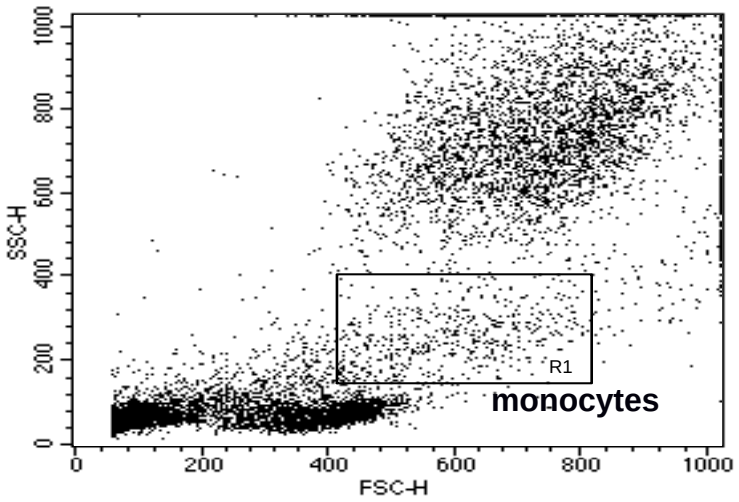
- CD14+CD16+ monocytes have high capacity to produce inflammatory cytokines (TNF-alpha, IL-6, and IFN-α) and promote inflammation

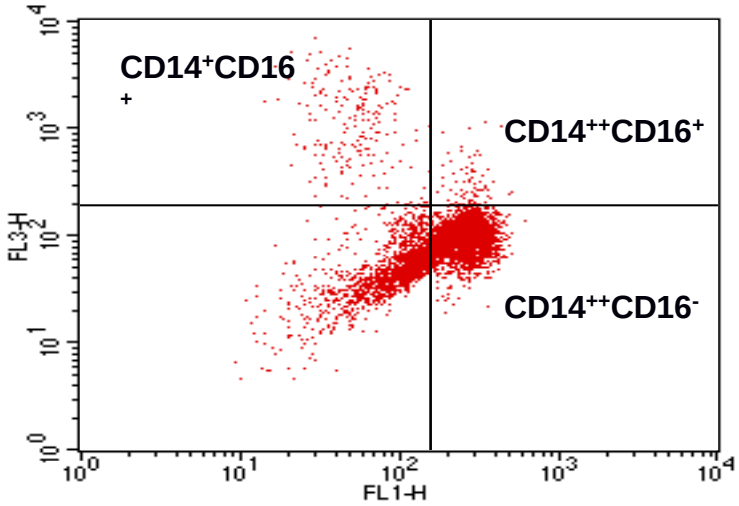
Impact of ESRD on Monocytes

- General expansion, particularly of CD14+CD16+ subset
- Elevated basal expressions of Toll-like receptor (TLR)-2, TLR-4 & integrins
- Increased basal cytokines and ROS production
- Decreased phagocytic capacity

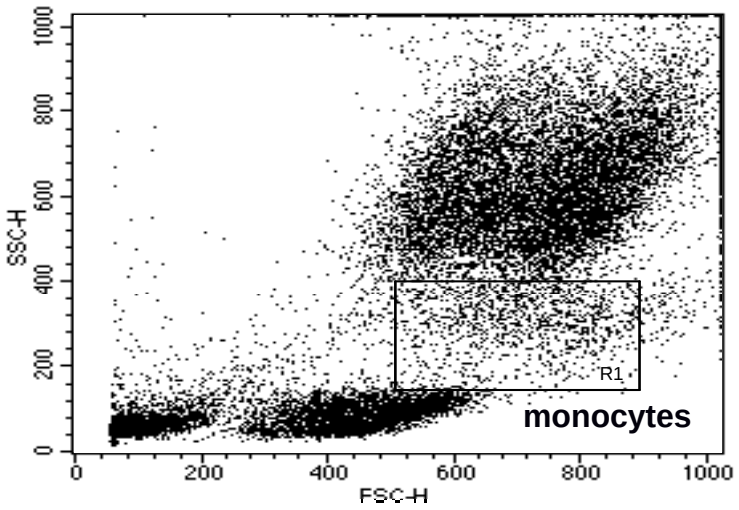
These abnormalities illustrate the contribution of monocytes to the prevailing inflammation & immune deficiency in ESRD

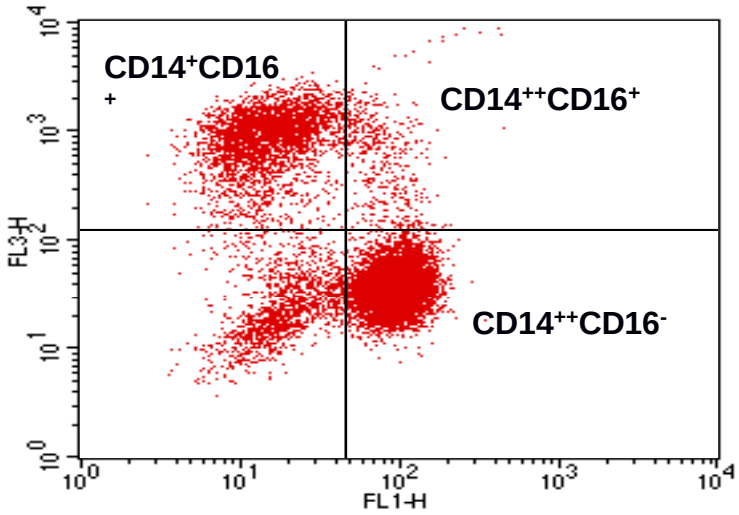
Contro



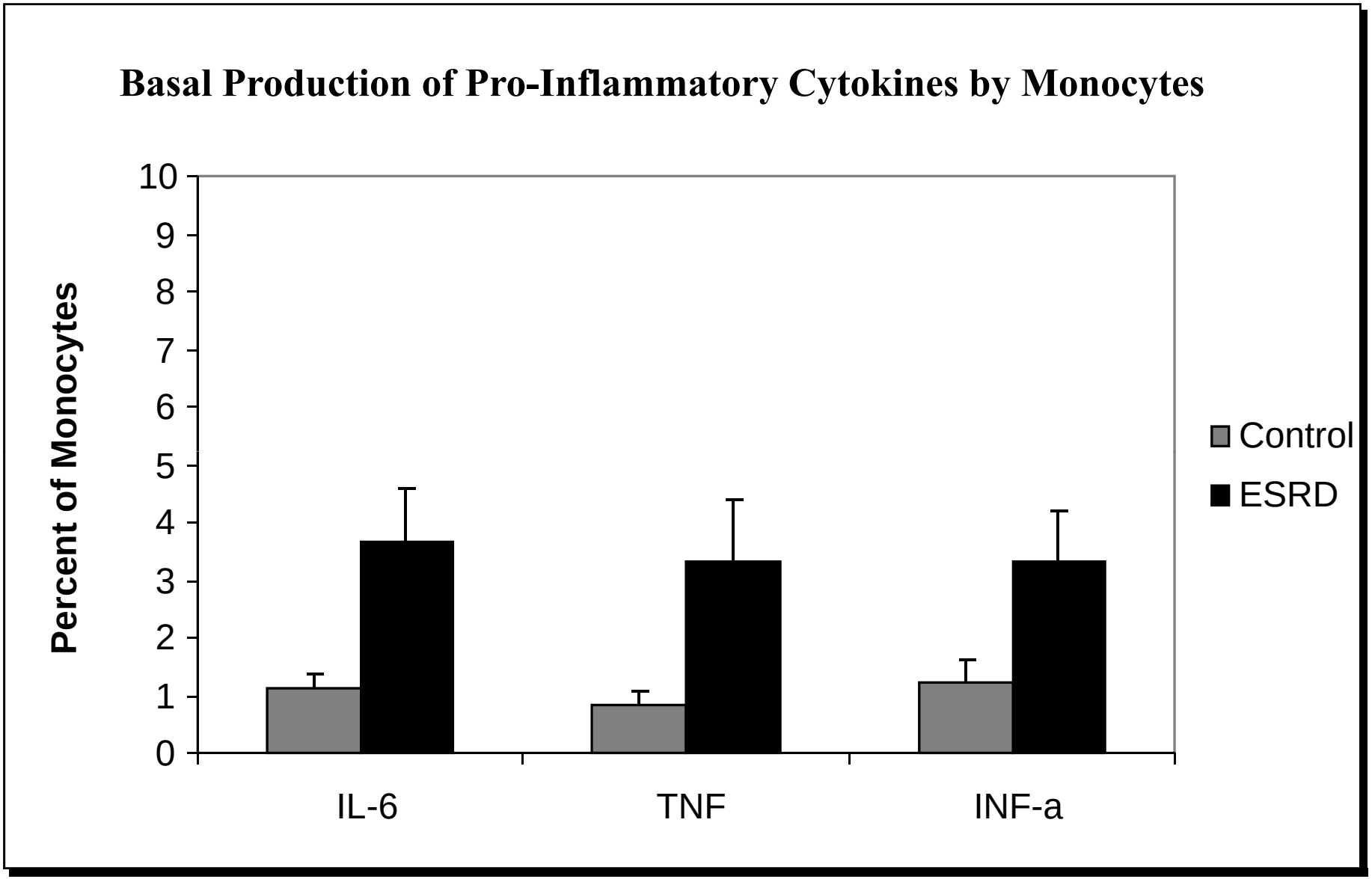


ESRD



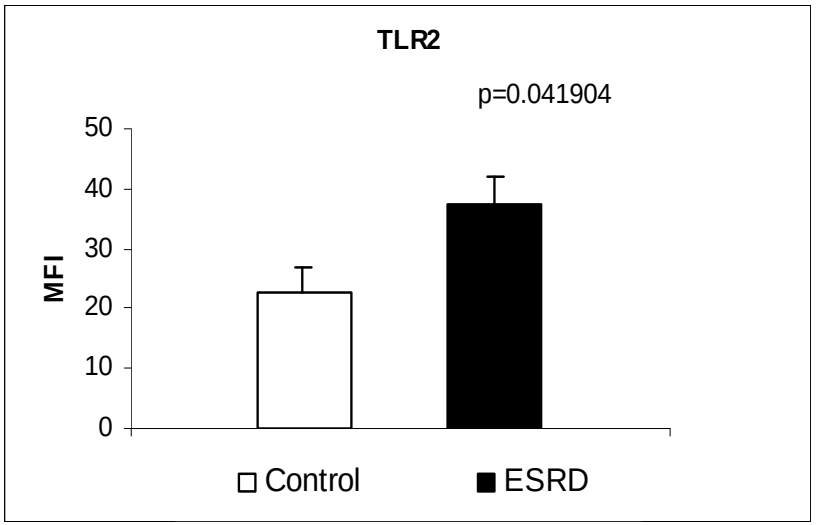


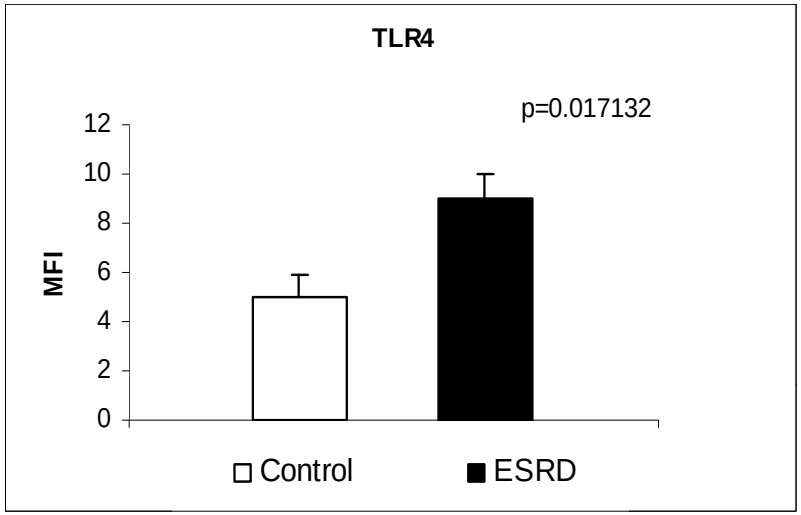
Increased number of $CD14^{+}CD16^{+}$ monocytes in an ESRD patient before hemodialysis.

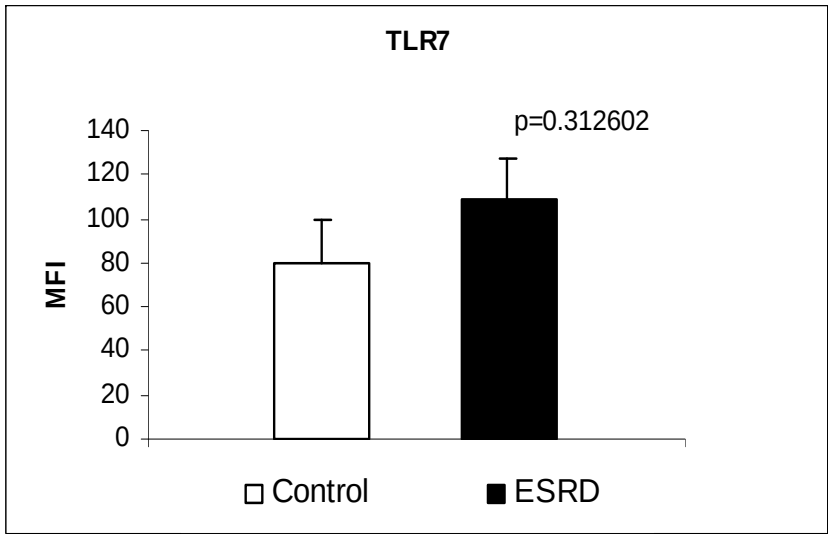


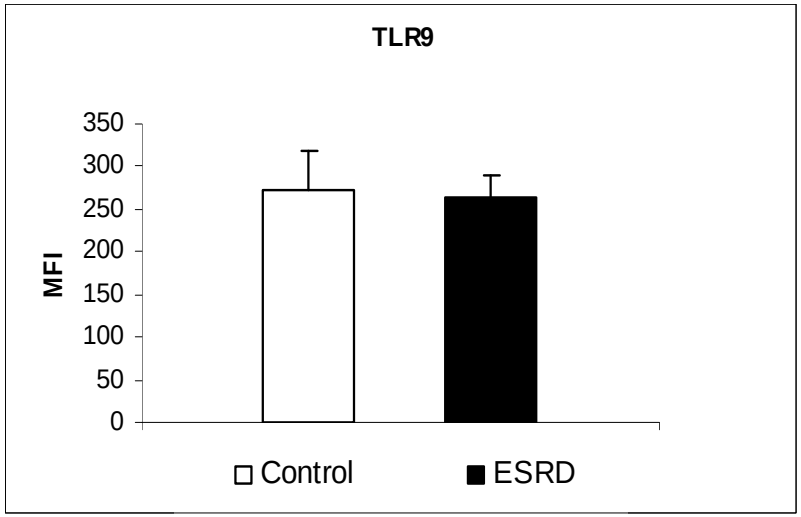
Percent of monocytes producing pro-inflammatory cytokines in controls vs. ESRD patients.

TLR expression in monocytes

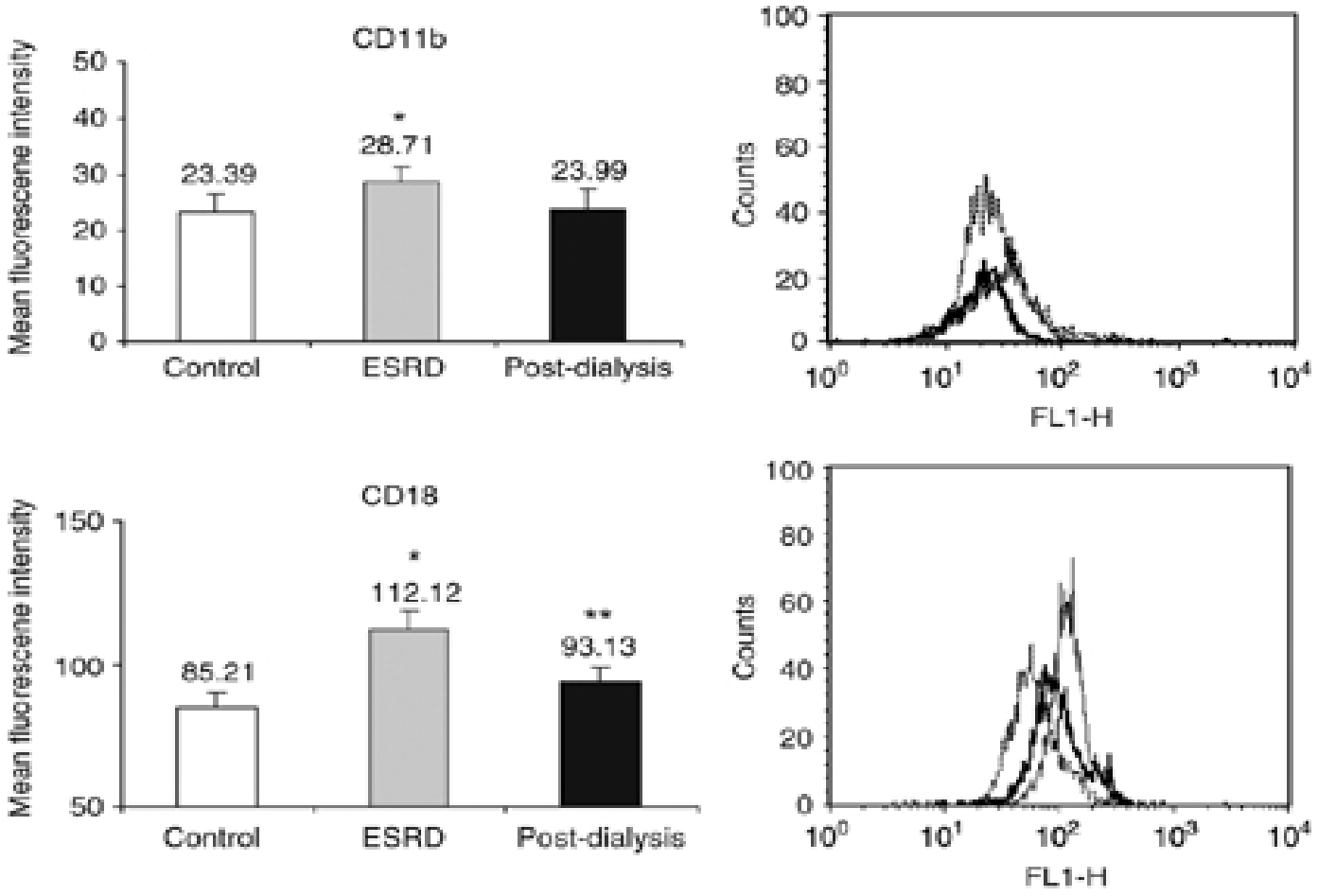








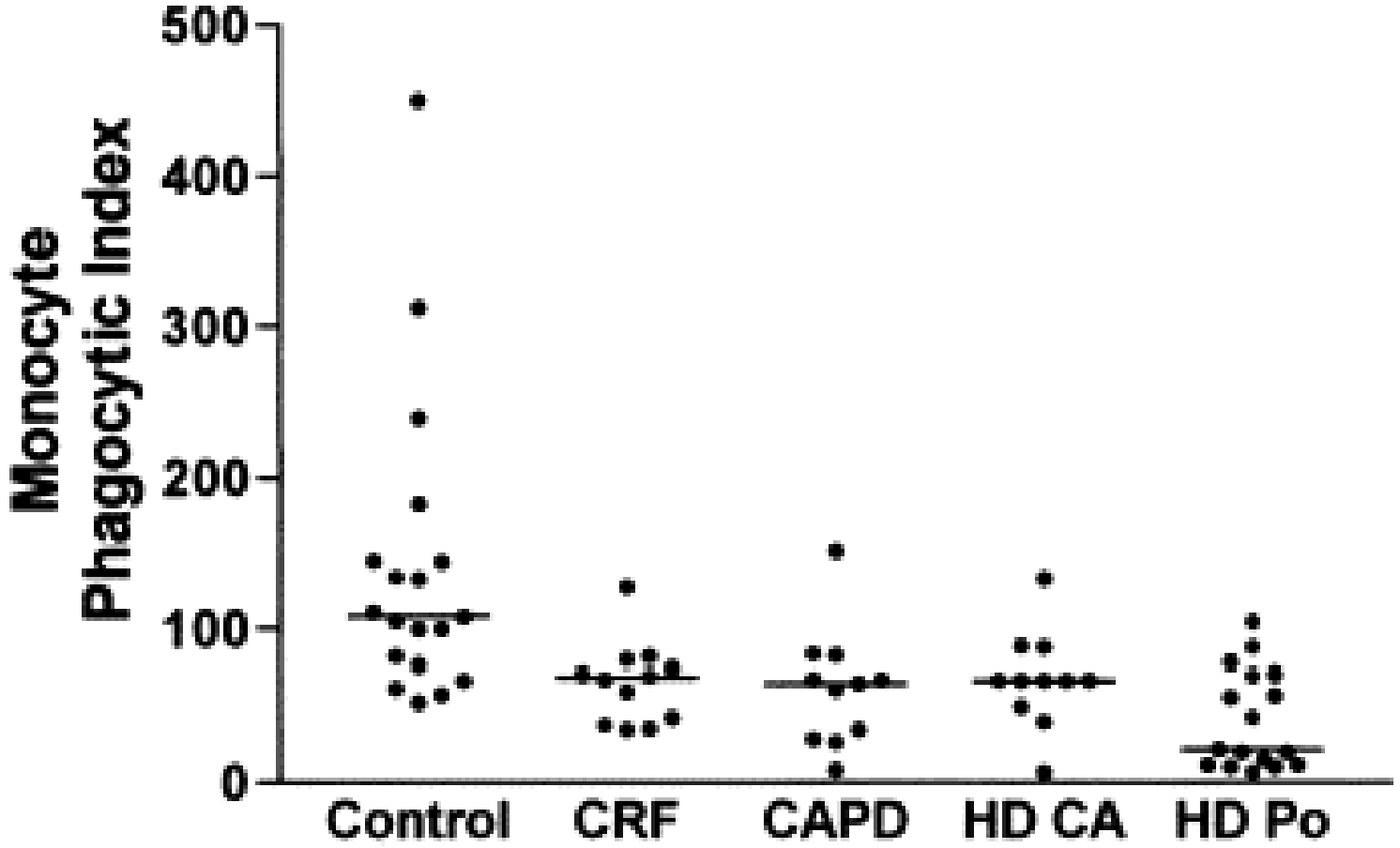
Monocyte Integrin Expression



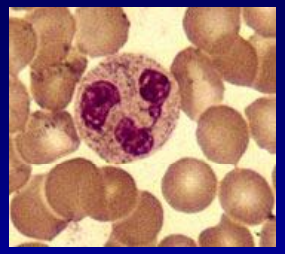
*P<0.05 compared to control

**P<0.05 compared to pre-dialysis value

Monocyte phogocytic capacity in CKD



Muniz-Junqueira et al .Life Sci. 2005



Polymorphonuclear leukocytes

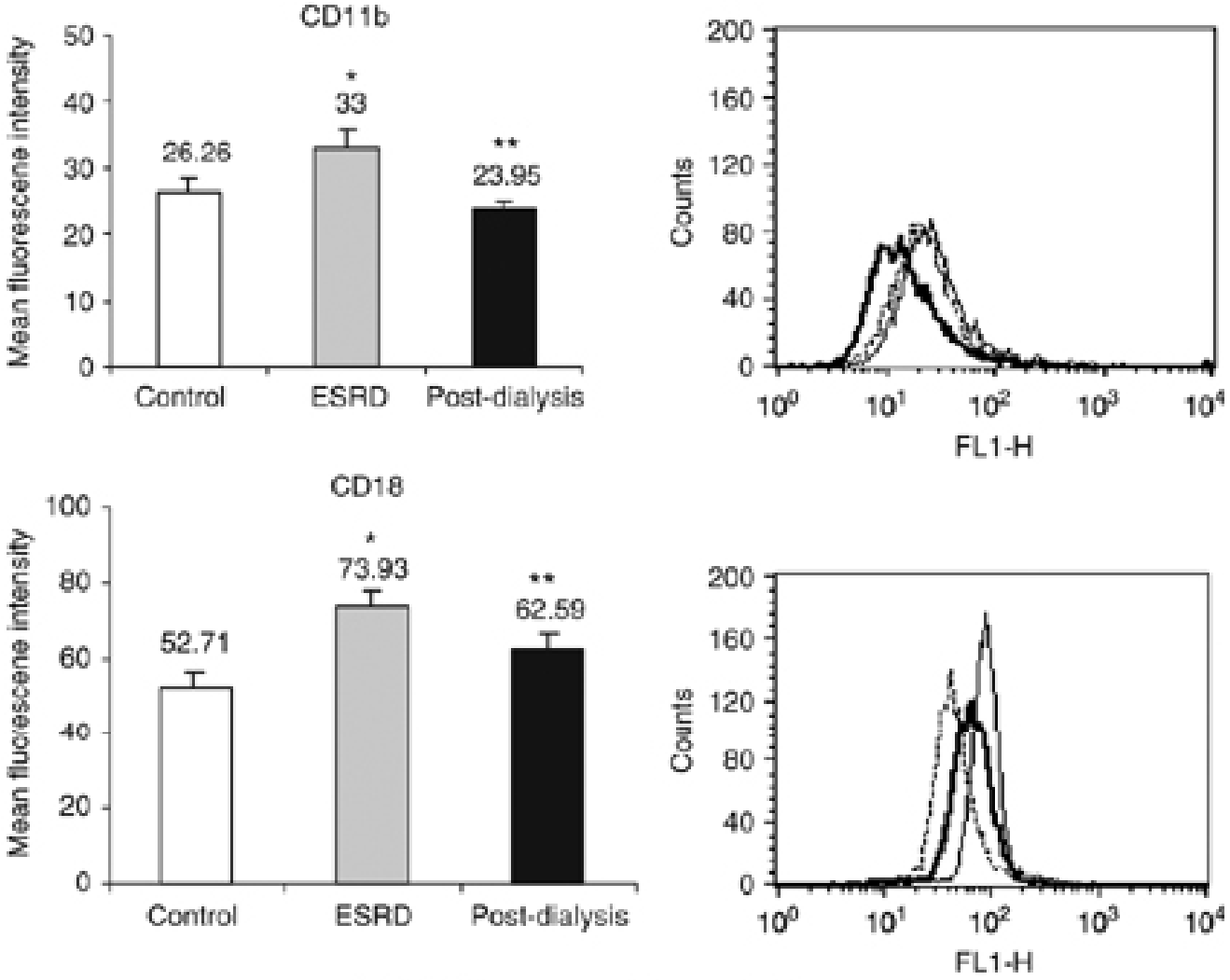
- Short-lived (5 days) professional phagocytes which avidly engulf antibody- & complement-coated microbes, damaged cells & cellular debris
- They have many intra-cellular granules which contain bacteriocidal proteins (cationic proteins and defensins), proteolytic enzymes & cathepsin G (to degrae bacterial proteins) & lysozyme (to lyse bacterial walls) as well as NOX-II & myeloperoxidase (to generate ROS) and lactoferin (to deprive bacteria from access to Fe)
- PMNs represent the first line of defense against invading microbes & are important player in inflammation

Impact of ESRD on Polymorphonuclear leukocytes

- Spontaneous PMN activation leading to:
  Increased integrin expression
  Degranulation
  Increased basal ROS production
- Decreased phagocytic & ROS production capacities
- Increased apoptosis

These events are transiently intensified by hemodialysis

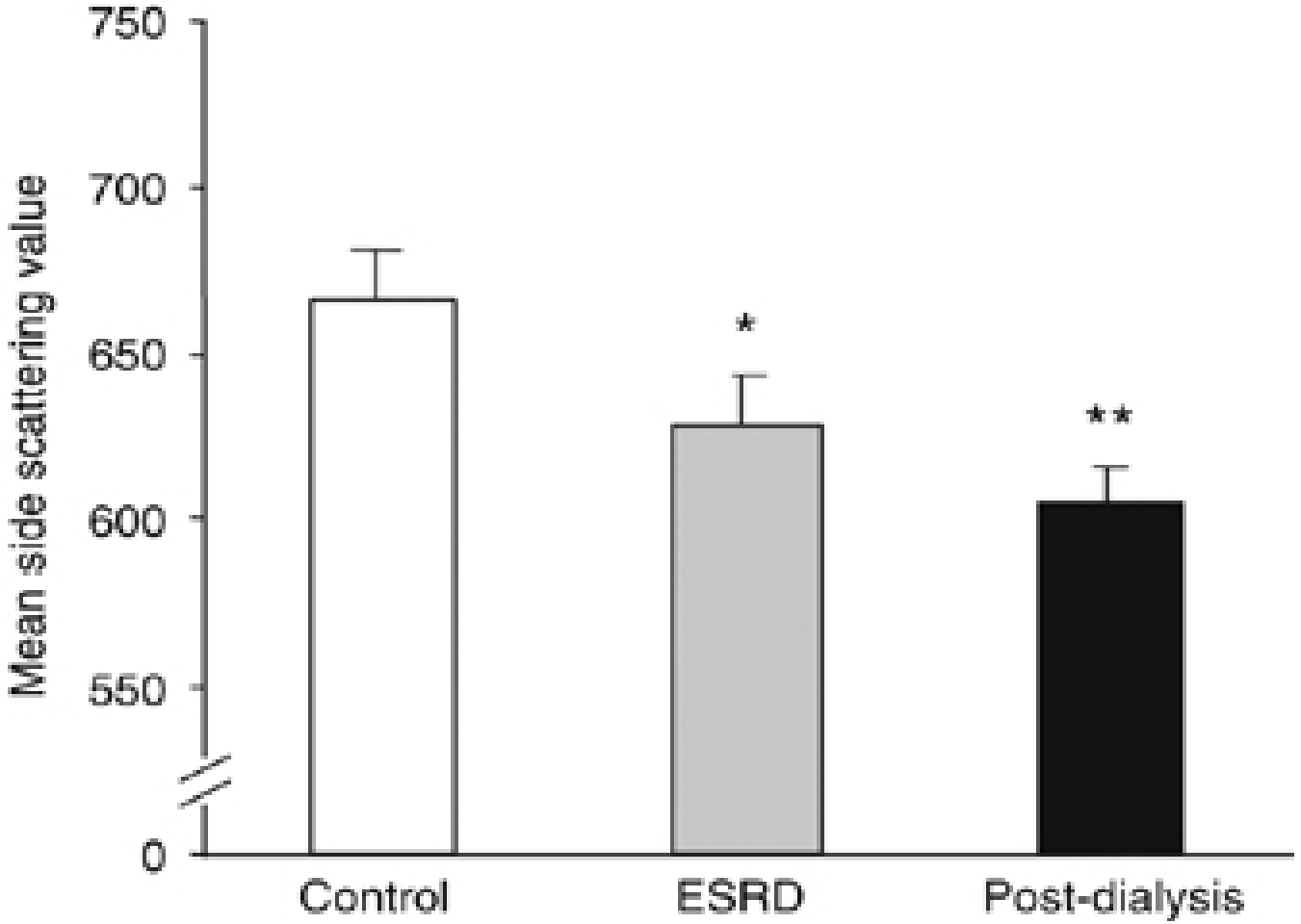
Integrin expression in circulating granulocyte



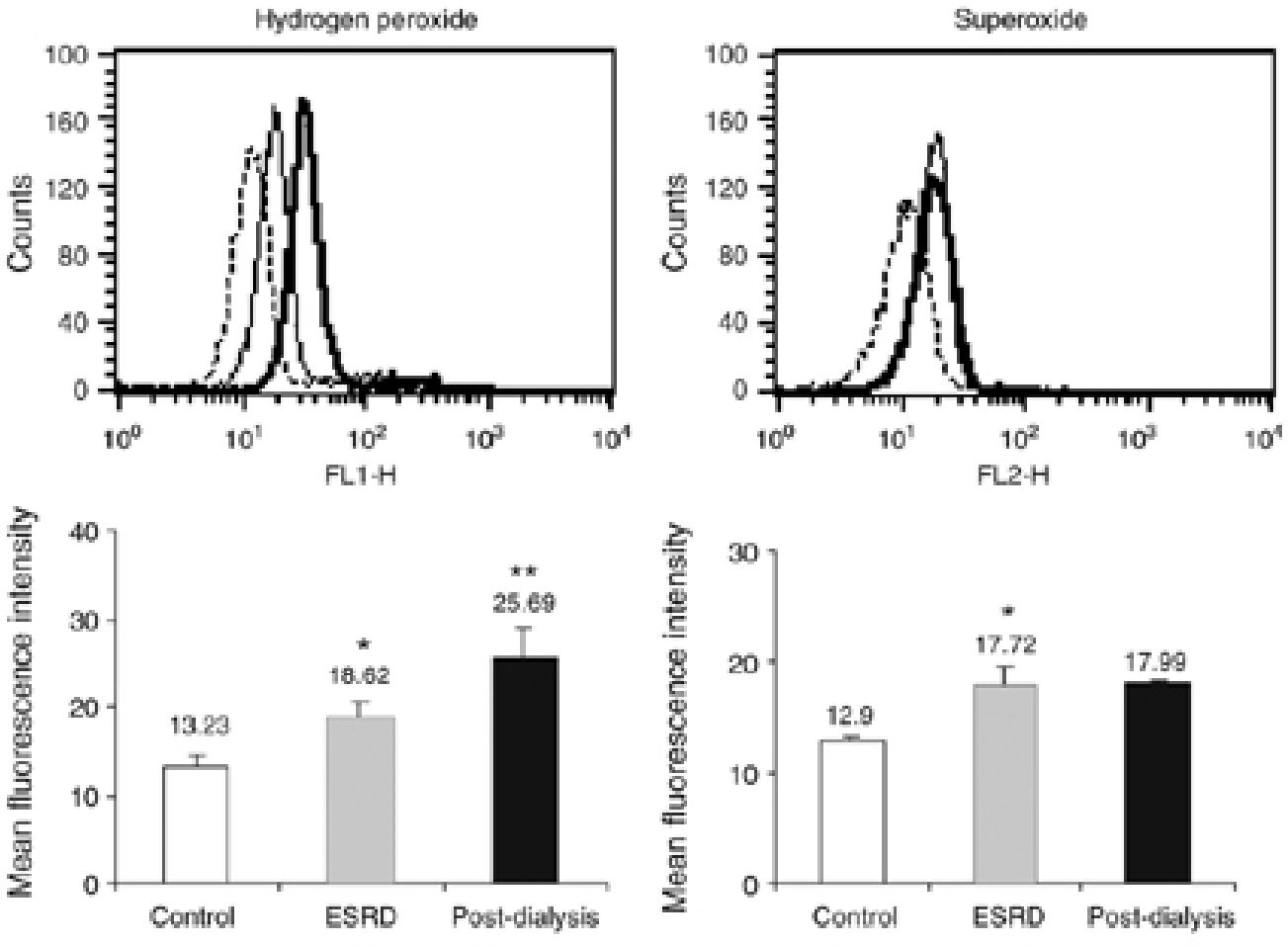
*P<0.05 compared to control

**P<0.05 compared to pre-dialysis value

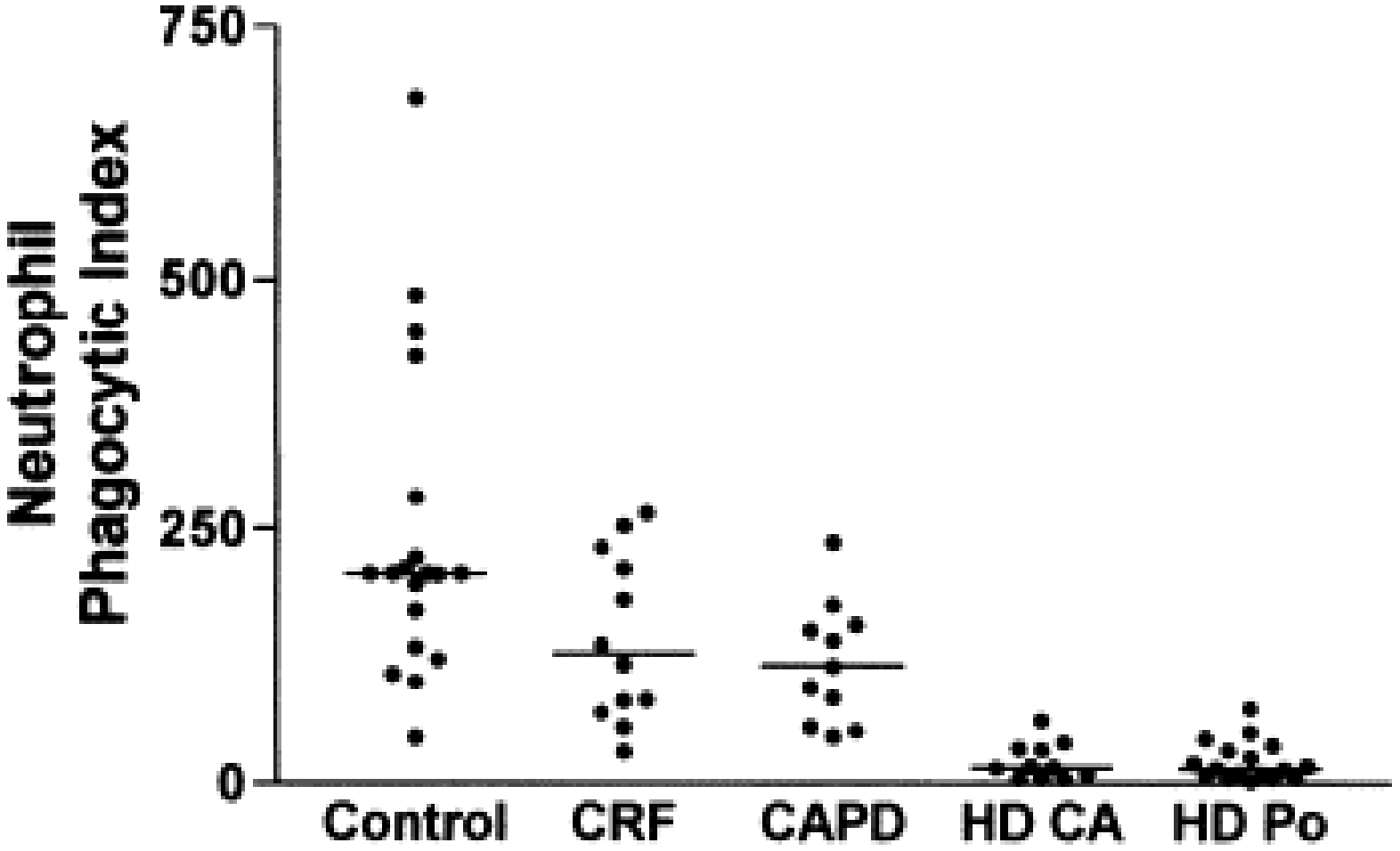
Effect of ESRD and hemodialysis on granularity of circulating granulocyte



Effects of ESRD and hemodialysis on superoxide and H_2O_2 Production in the circulating granulocyte



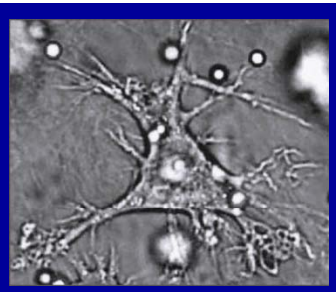
PMN phogocytic capacity in CKD



Muniz-Junqueira et al .Life Sci. 2005

Summary; monocyte & PMN abnormalities in ESRD

- Granulocyte and monocytes in ESRD patients exhibit spontaneous activation and ROS generation, increased apoptosis & reduced phagocytic ability
- Studies in experimental animals with CKD have shown upregulation of ROS production machinery & chemokine expression in the cellular constituents of various tissues highlighting their participation in the prevailing inflammatory state
- These observations point to simultaneous activation & diminished capacity of innate immune system in ESRD, leading to oxidative stress, inflammation and impaired defense against microbial infections



Dendritic cells

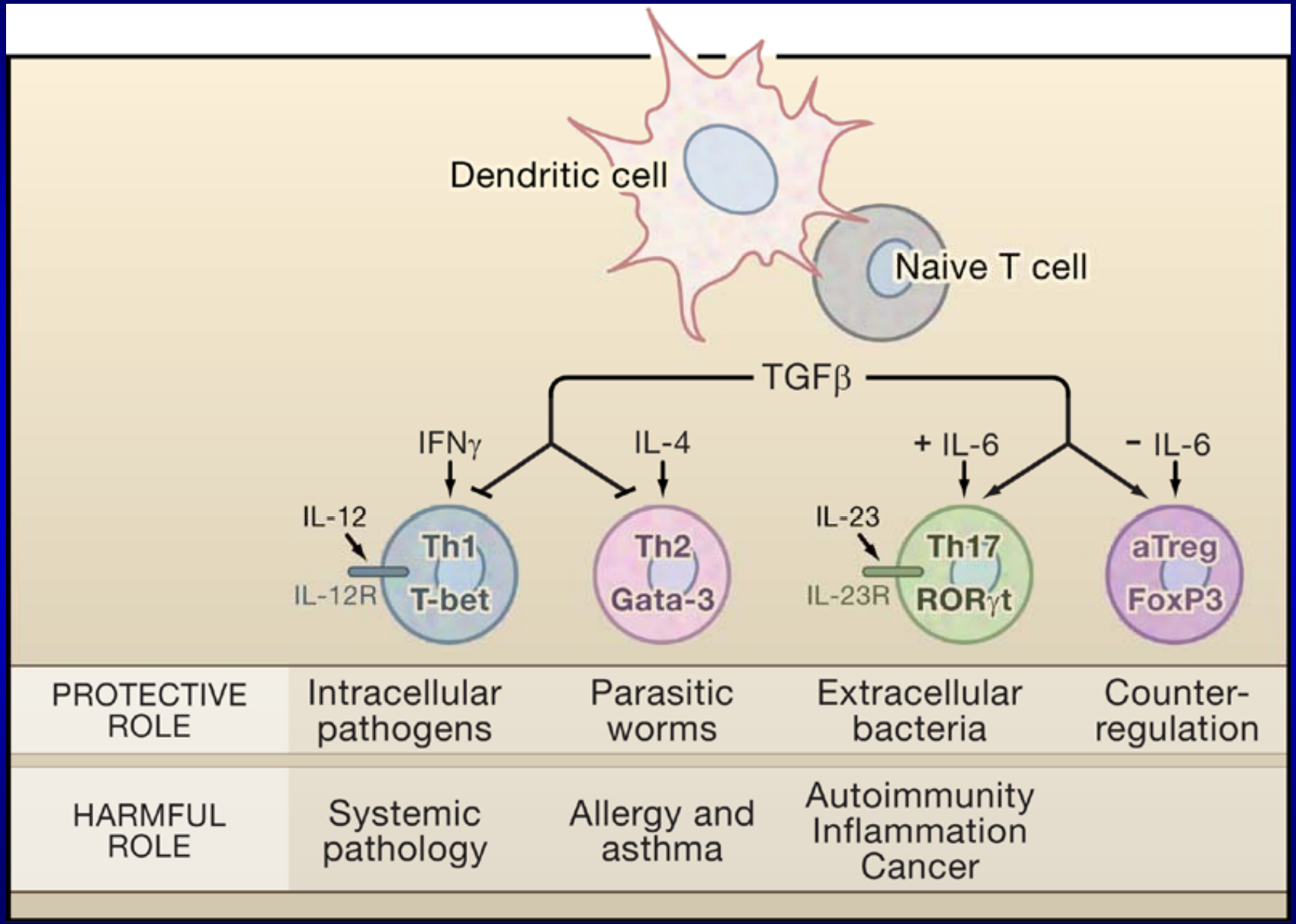
- Dendritic cells (DCs) are the major antigen presenting cells that play a key role in initiation and maintenance of innate and adaptive immunity.

- They continuously survey antigenic milieu and act as the sensors of microbial invasion or tissue damage

- Activation of DCs by pathogens triggers secretion of cytokines and upregulation of co-stimulatory molecules which are essential for priming of the naïve T cells to the captured antigens

- DCs serve as the sentinel cells for adaptive immunity by regulating immune responses in T cell, B cell and natural killer cells and thereby play a critical part in tolerance to self antigens, tumor surveillance and defense against microbial pathogens

Helper T cell differentiation in lymph node



PROTECTIVE ROLE	Intracellular pathogens	Parasitic worms	Extracellular bacteria	Counter-regulation
HARMFUL ROLE	Systemic pathology	Allergy and asthma	Autoimmunity Inflammation Cancer	

Adapted from SL Reiner, Cell 129:33-36, 2007

Dendritic Cell subtypes

Plasmacytoid Dendritic Cell (pDC)

- Contain intracellular TLR7 and TLR9 which sense viral or self nucleic acids (autoimmune diseases)
- Produce large amounts of type I interferons e.g. IFNα in response to viral infections

Myeloid Dendritic cell (mDC)

- Possess cell surface TLRs including TLR3 and TLR4
- Produce IL-12 and type I interferons favoring Th1 differentiation

Effect of ESRD on Dendritic Cells

- ESRD results in dendritic cell (DC) depletion which is primarily due to the reduction of pDC subset
- Depletion of circulating DCs is transiently exacerbated by hemodialysis procedure
- Given the critical role of DCs in regulation of innate and adaptive immunity, DC depletion must contribute to impaired defense against microbial infections and poor response to vaccination in ESRD population
- Basal and LPS-stimulated TNF production by DCs is increased in ESRD, suggesting a potential role in the pathogenesis of uremia-associated inflammation

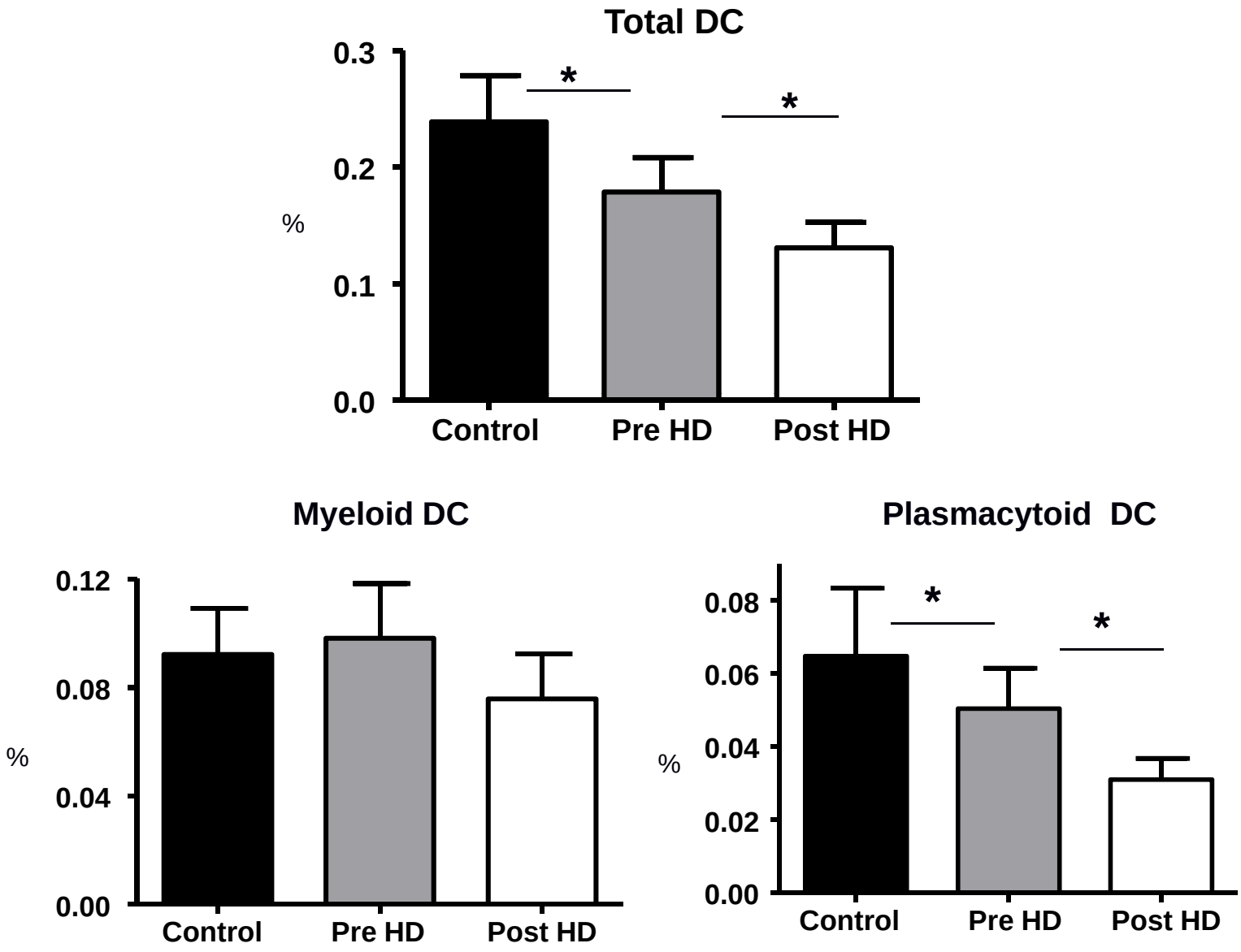

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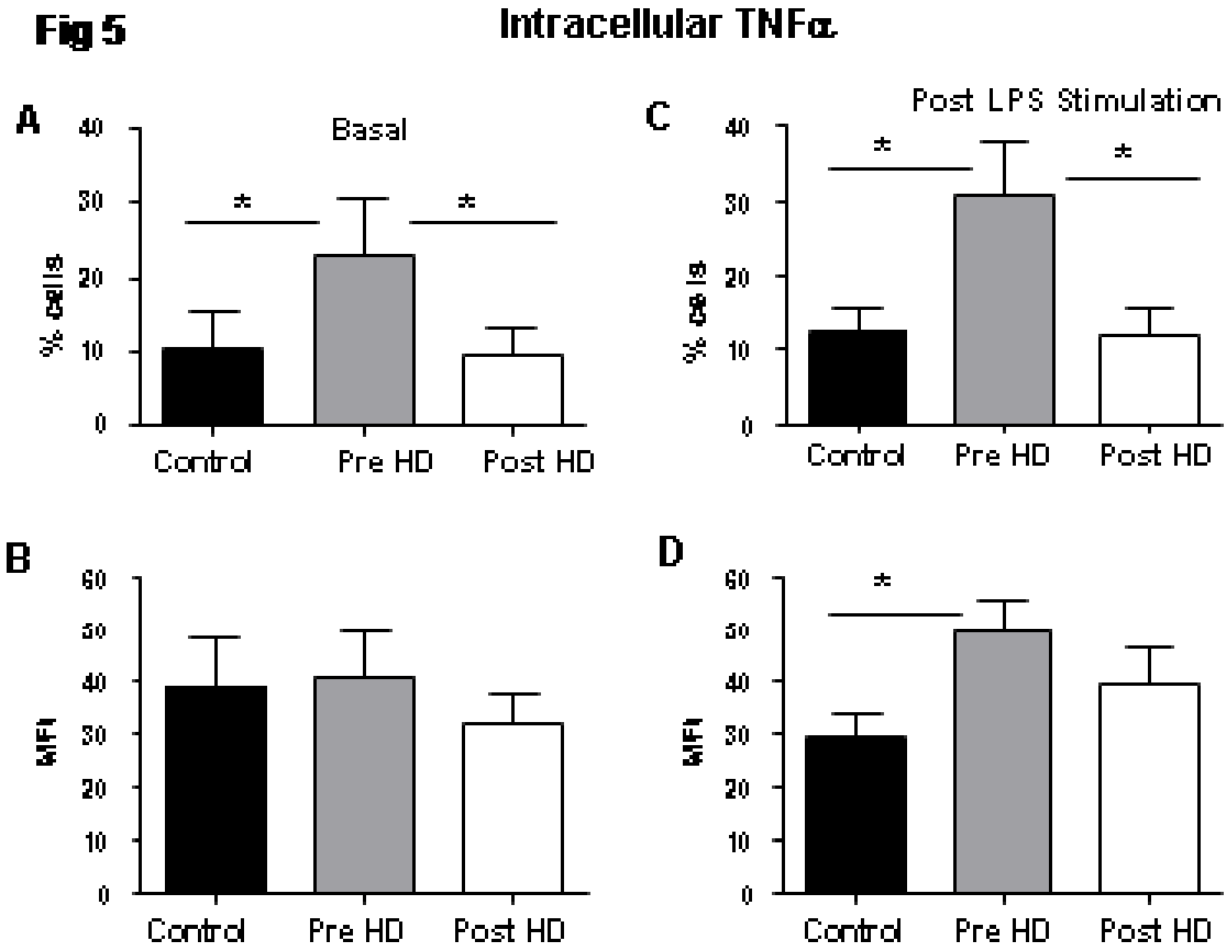
Fig 5


Intracellular TNFα
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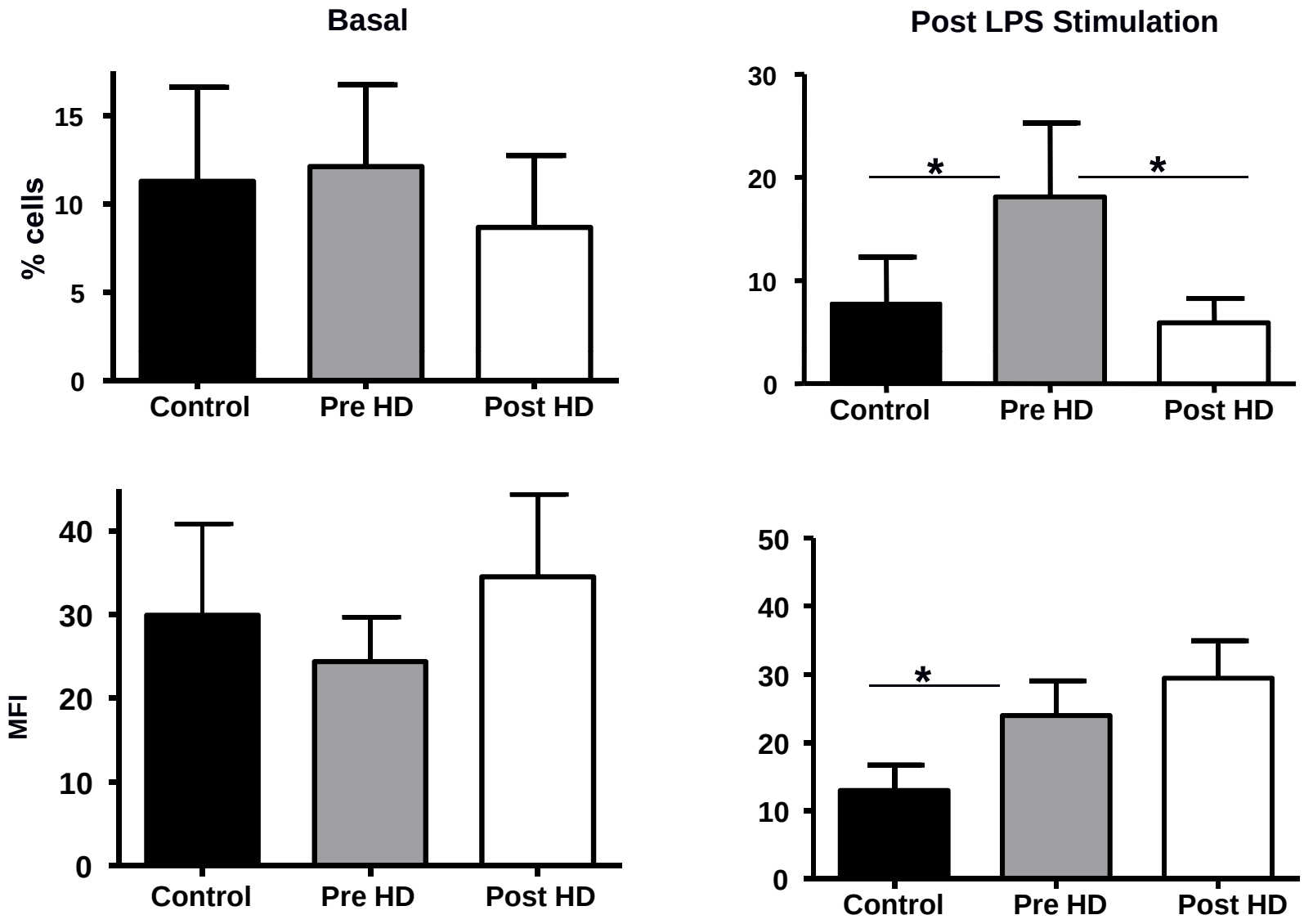
Intracellular IFNα



Effect of ESRD on the agents of Adaptive Immunity

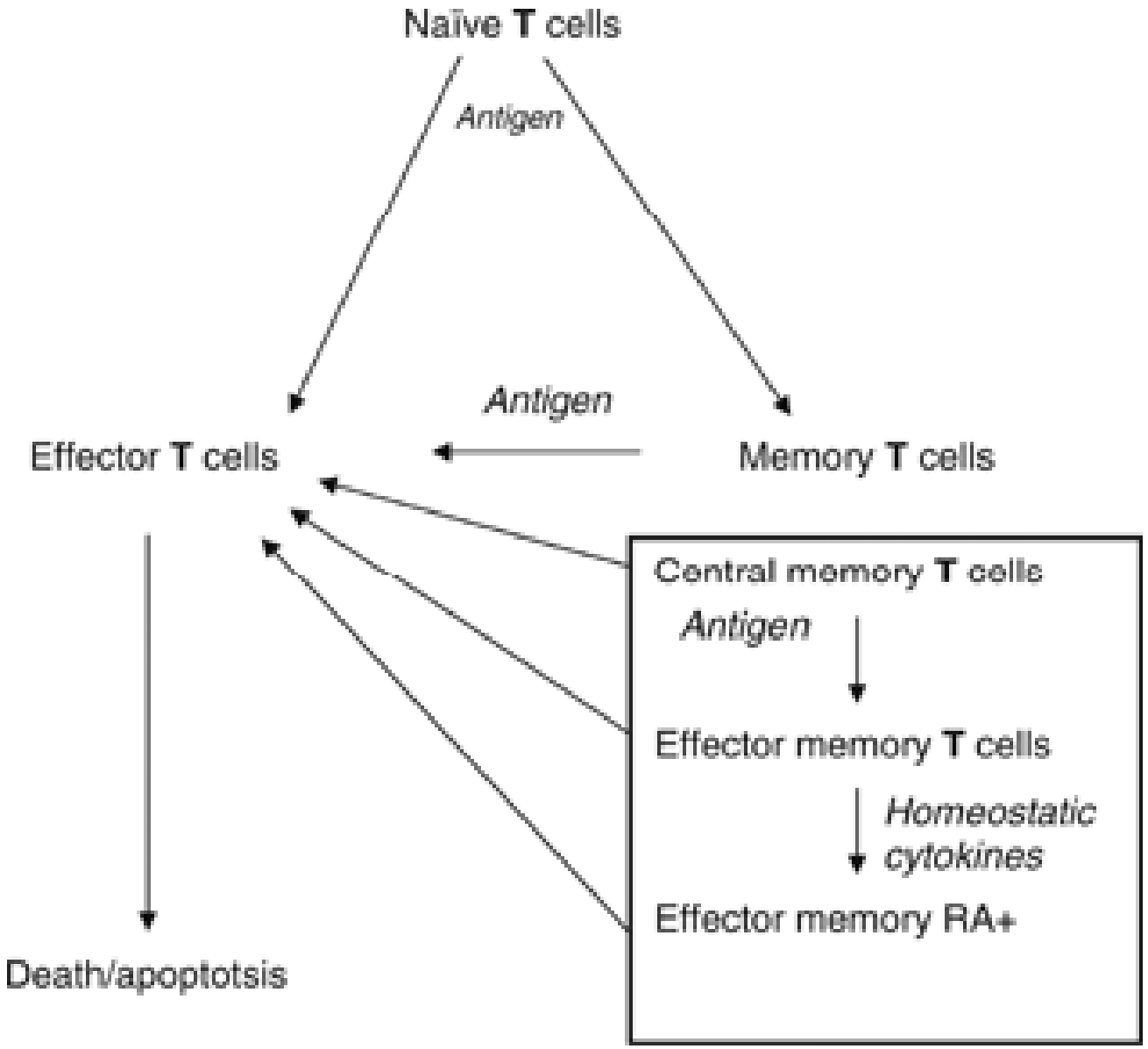
- T cell depletion/dysfunction
- B cell lymphopenia

T lymphocytes

- **Helper (CD4+) T lymphocytes help:**
  - **B cells to produce antibody**
  - **Recruit PMNS, eosinophils & basophils to the cites of infection/inflammation**
  - **Increase microbicidal activity of Macrophages**
  - **Promote tolerance or suppress inflammation**

- **Cytotoxic (CD8+) T lymphocytes**
  - **destroy virally infected and tumor cells**
  - **participate in transplant rejection**
  - **can be transformed to an anergic state by T regulatory cells**

T lymphocytes



Helper T (Th) cell subsets

Products of alternative differentiation of naïve cells

- Th1
- Th2
- Th17
- aTreg (adapted regulatory T cells)

Emerging from thymus as distinct lineage

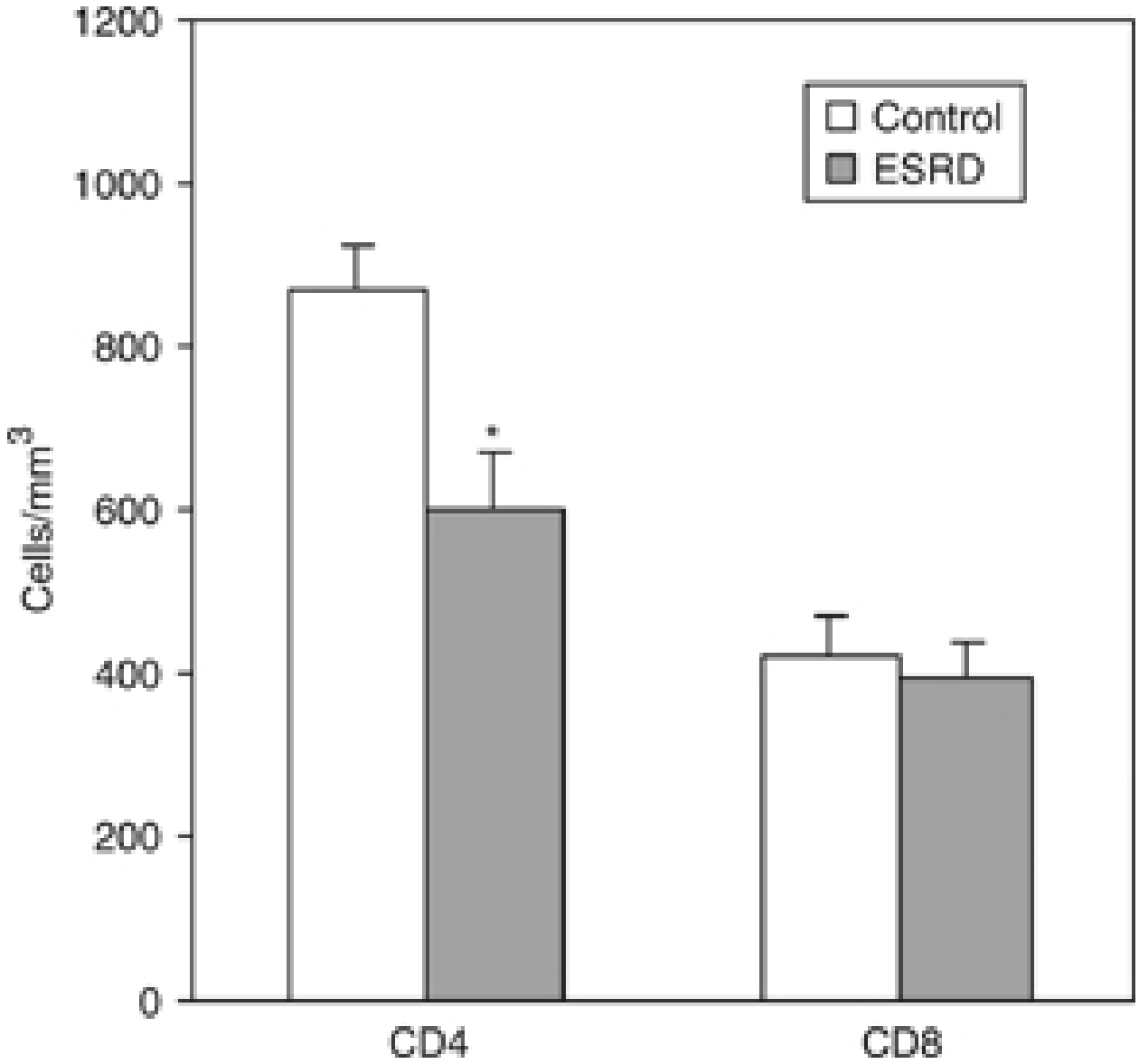
- Natural killer cells (NTK)
- Natural regulatory T cells (nTreg)

Effects of ESRD on T lymphocytes

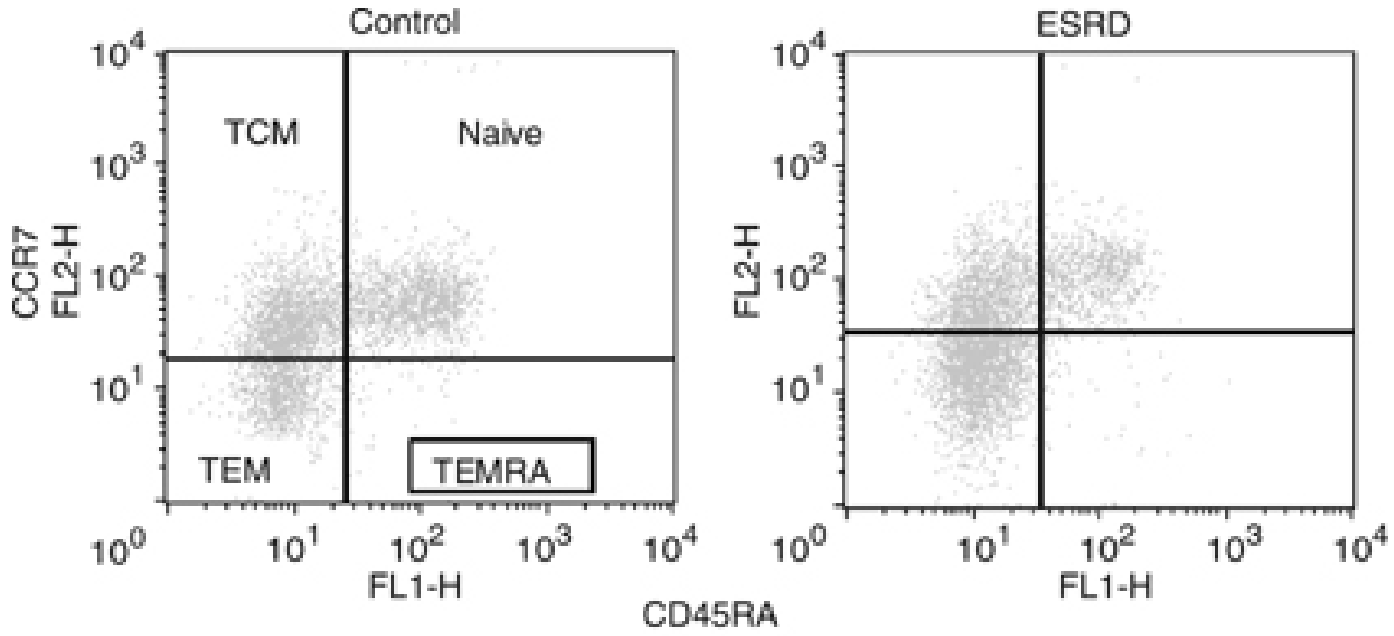
- Reduced CD4/CD8 ratio
- Increased Th1/Th2 ratio
- Depletion of naïve and central memory T cells.

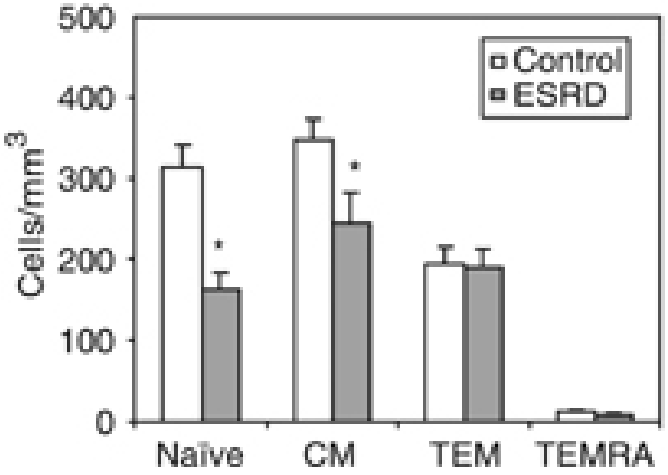
Given the critical role of naïve and central memory T-cells in orchestrating the immune response to the *de novo* exposure or re-exposure to pathogens, their depletion must be, in part, responsible for increased incidence and poor outcome of various infections in ESRD population

Helper (CD4+) and cytotoxic (CD8+) T lymphocyte subsets in ESRD

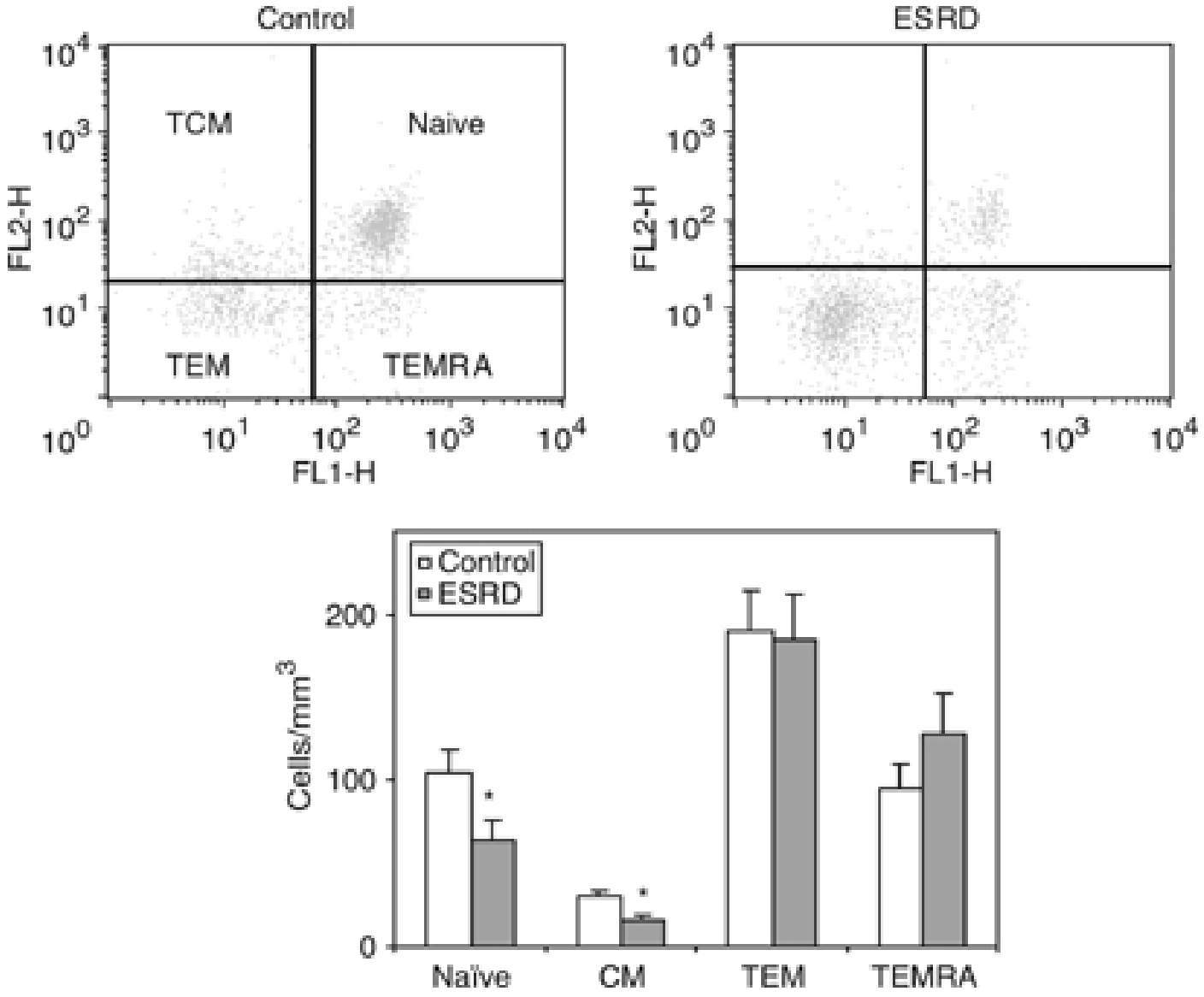


Helper T lymphocyte (CD4) subsets in ESRD

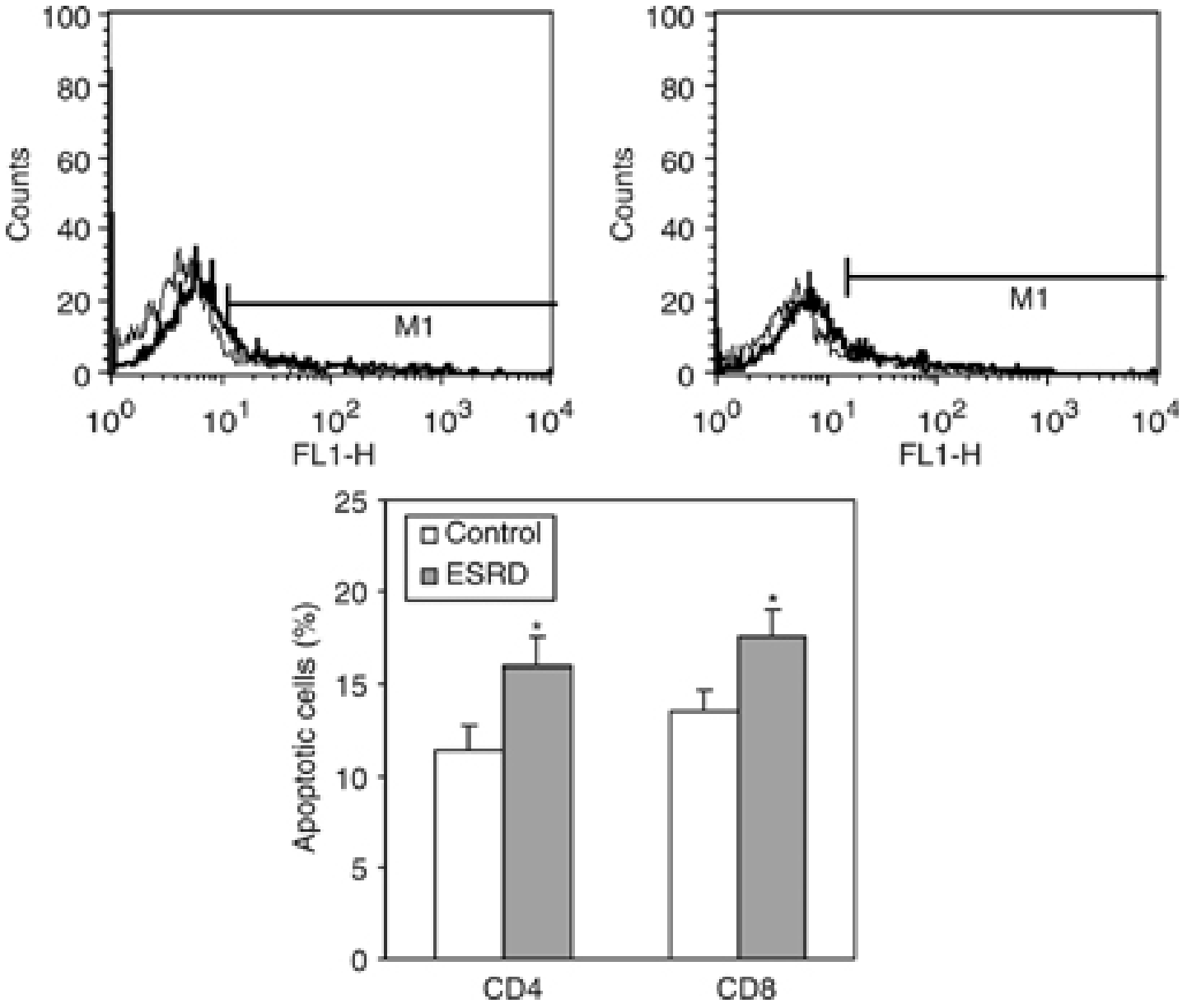




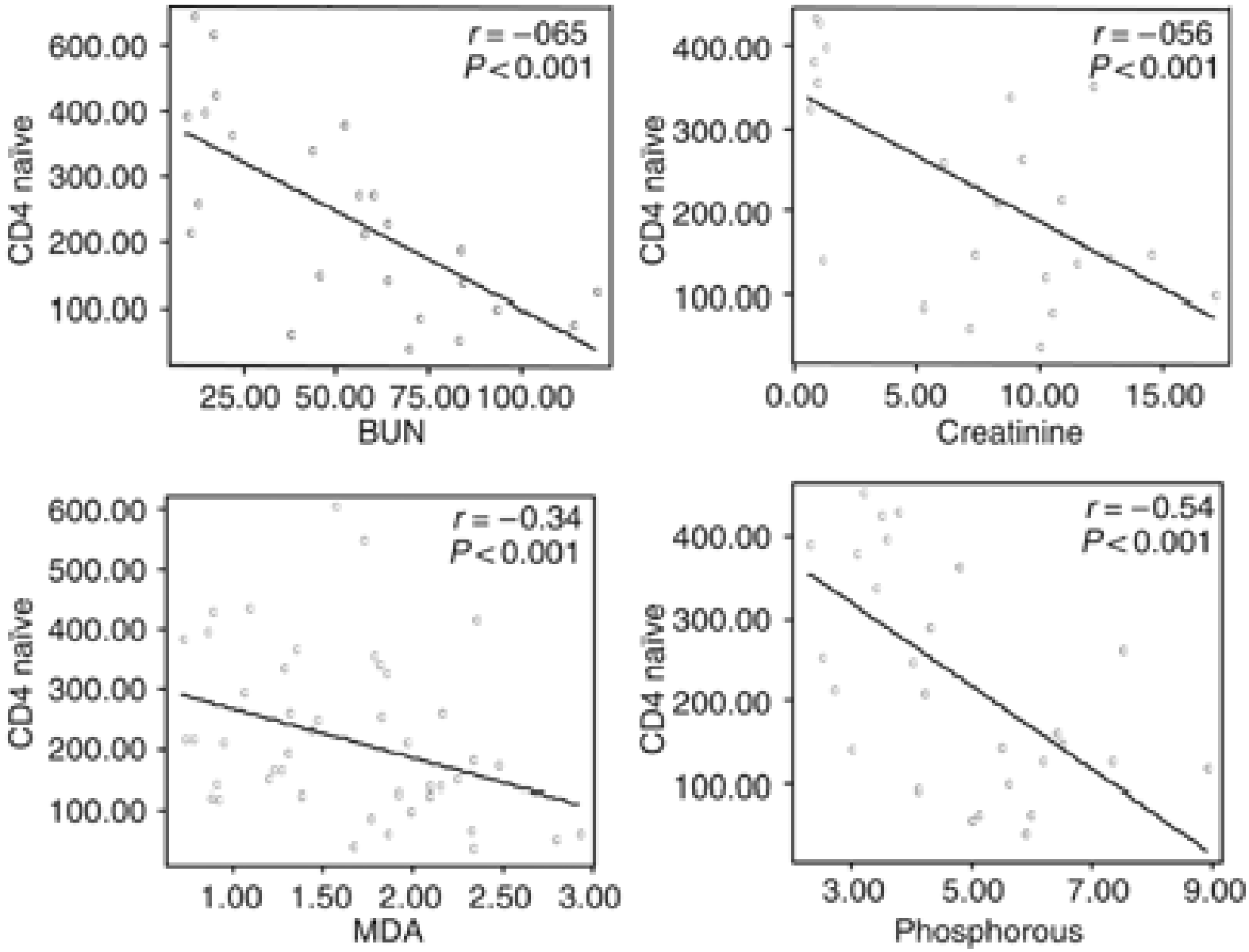
Cytotoxic T lymphocyte (CD8) subsets in ESRD

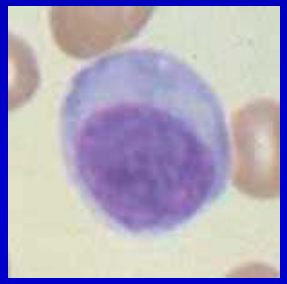


ESRD increases naïve and central memory T cell apoptosis



Naïve and CM T cell apoptosis is associated with oxidative stress & severity of uremia, and hyperphosphatemia





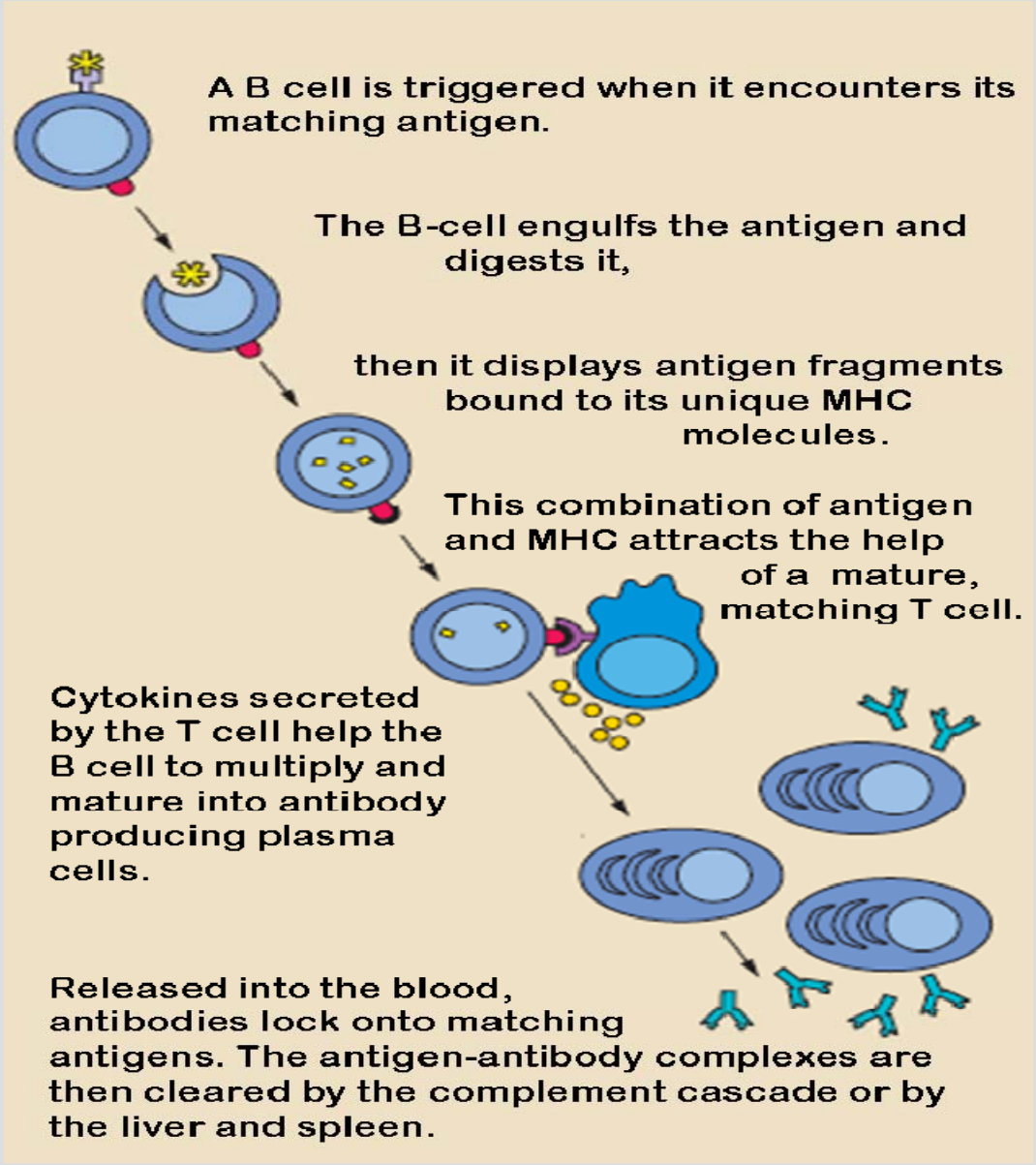
B lymphocytes/plasma cells

B cell function

. B cells conduct immune surveillance in blood & lymphatic system

- Each B cell has a unique receptor (BCR) which is made of a membrane-bound immunoglobulin molecule that can bind a specific antigen
- Once a B cell encounters its cognate antigen and receives an additional signal from a T helper cell, it differentiate into plasma cells and memory B cells
- Most activated B cells differentiate into plasma cells that secrete antibodies against the specific epitope of the inciting antigen
- A small minority survives as *memory cells* that recognize the given antigen. However, with each re-exposure, the number of surviving memory cells rises and specificity of immune response improves

B cell activation



Circulating B cell classification

Peripheral blood B cells (CD19+) are comprised :

- **newly formed transitional B cell ($_{CD19+,CD10+,CD27-}$)**
- **innate B1 cells (CD19+, CD5+)**
- **conventional B2 cells (CD19+, CD5−)**
- **naive B cells (CD19+, CD27−)**
- **memory B cells (CD19+, CD27+)**

Effect of ESRD on B Cells

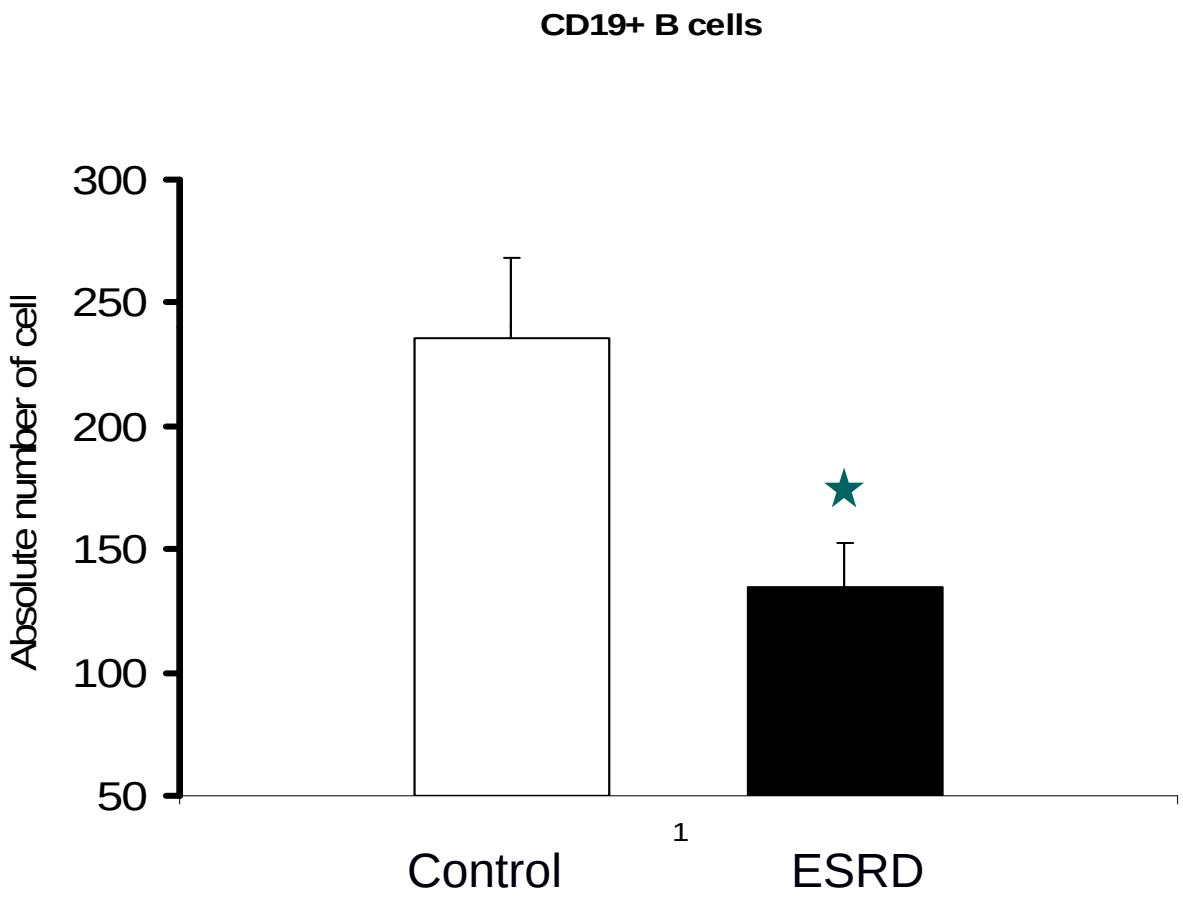
- ESRD causes diffuse reduction of B cell subpopulations

- B cell lymphopenia occurs despite elevated B cell growth (IL-7) and survival/differentiation (BAAF) factors

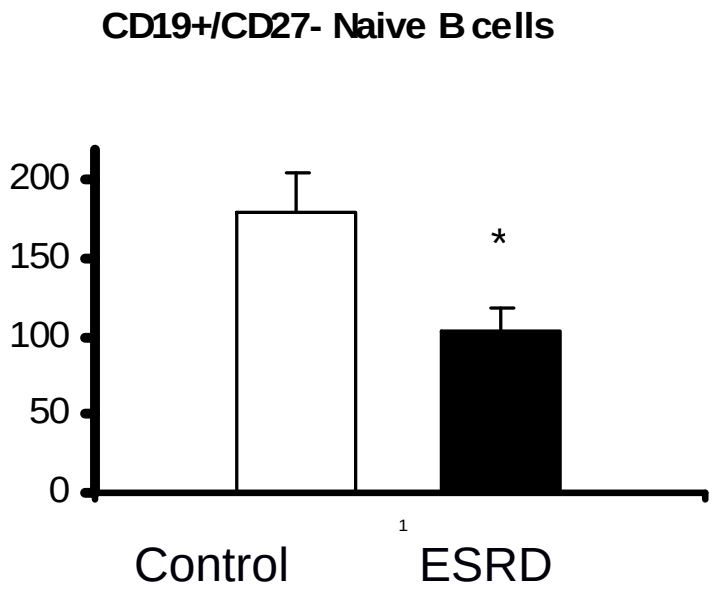
- This is associated with down-regulation of BAFF receptor in transitional B cells. This may contribute to B cell lymphopenia by promoting resistance to the actions of BAFF which is the potent B cell survival factor

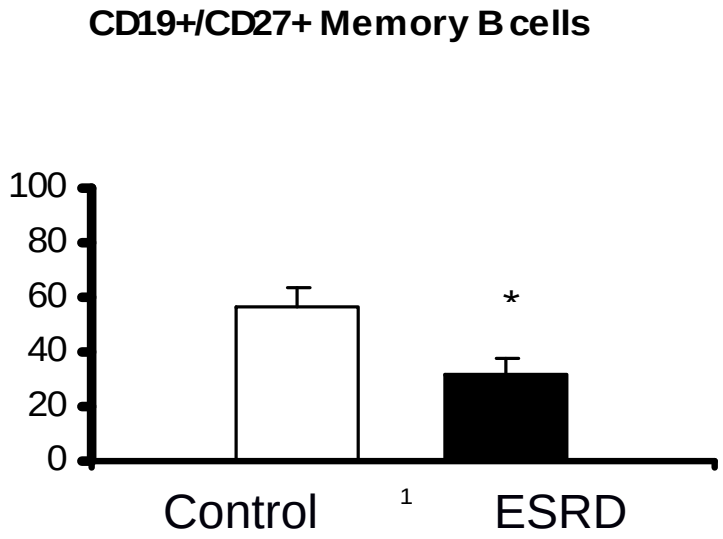
- The associated B cell lymphopenia can, in part, account for impaired response to vaccination and depressed adaptive immunity in ESRD population

Total B cell count



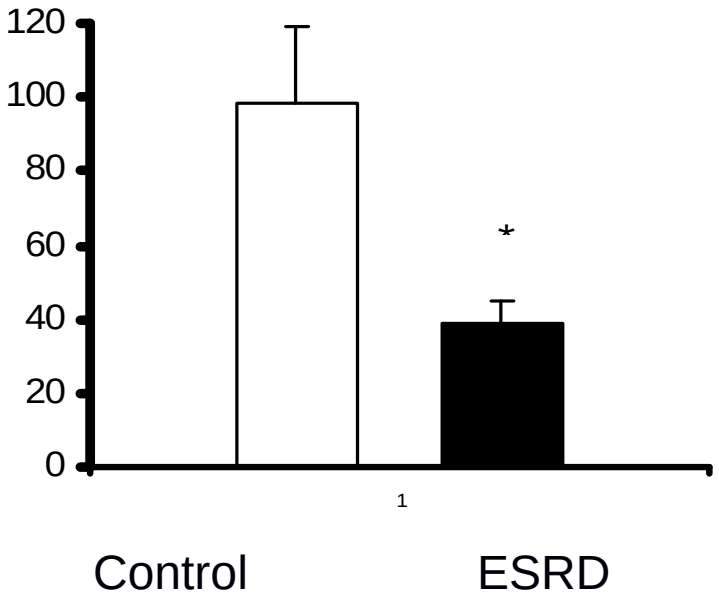
Naïve and central memory B cell depletion in ESRD



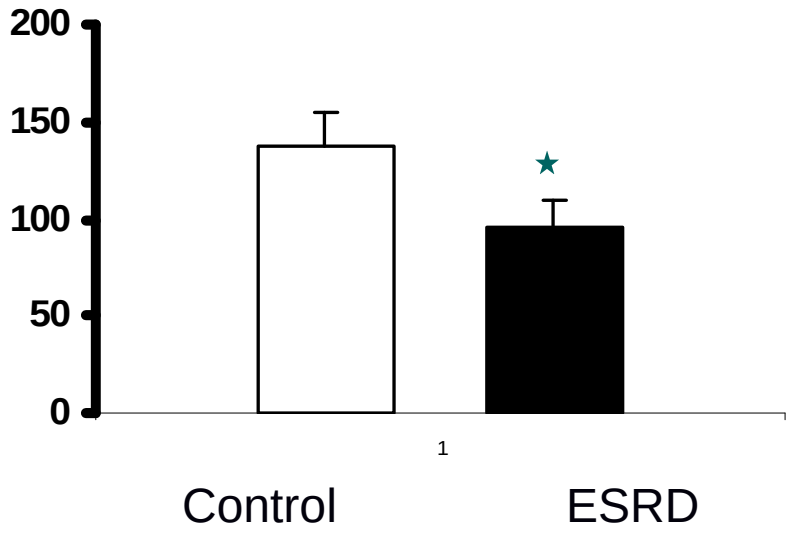


Innate and conventional B cell depletion in ESRD

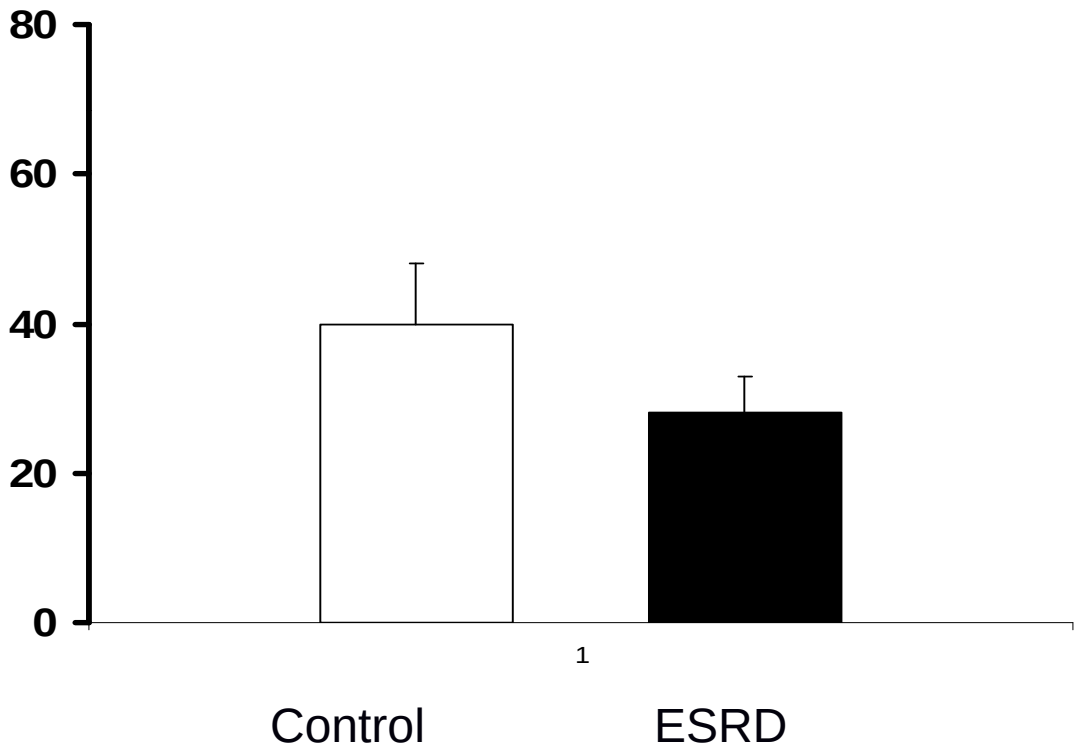
CD19+/CD5+ Innate(B1) B cells

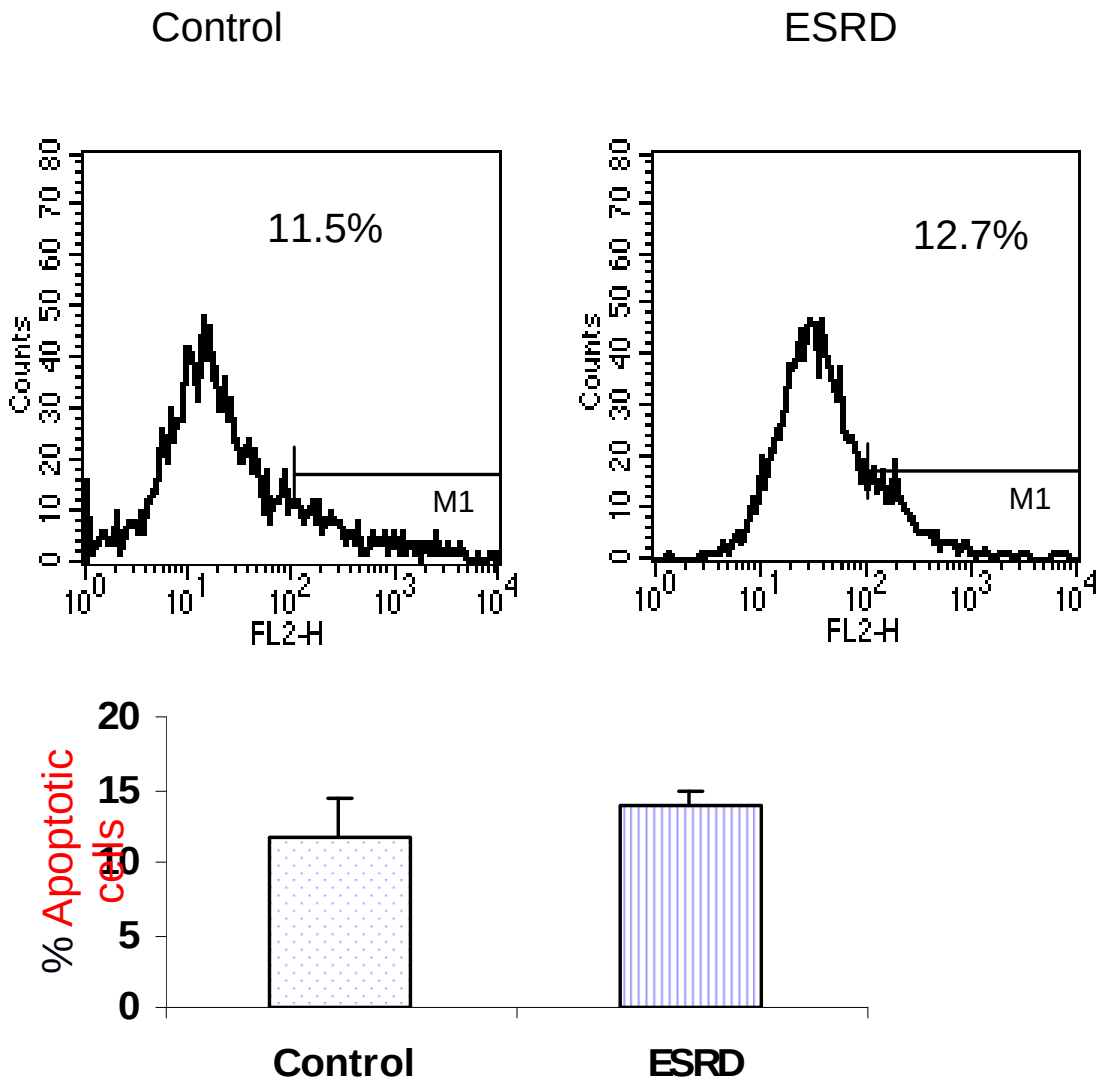


CD19+/CD5- Conventional (B2) B cells



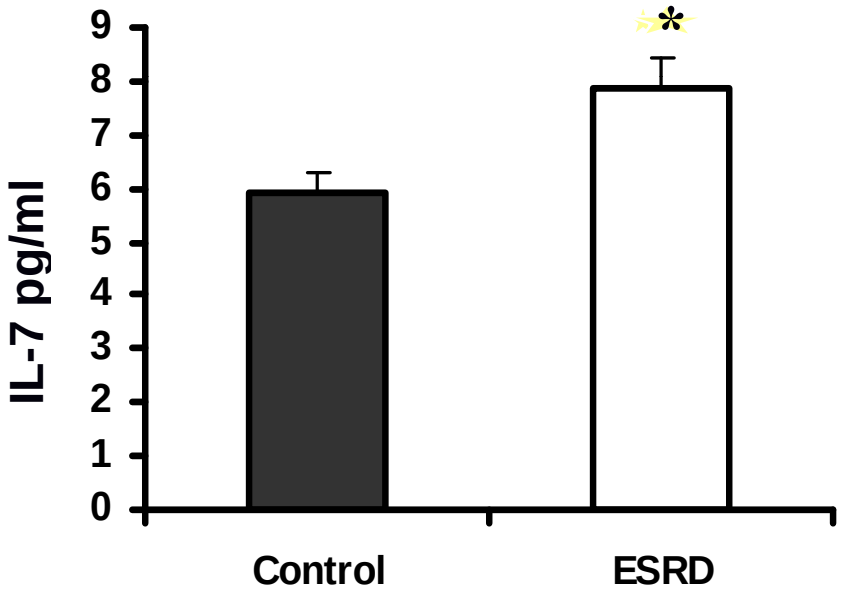
CD19+/CD10+ Transitional B cells

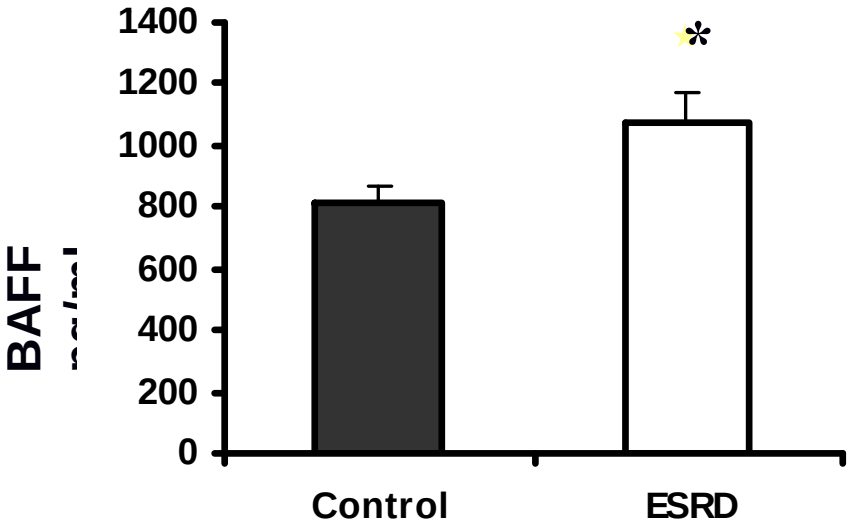




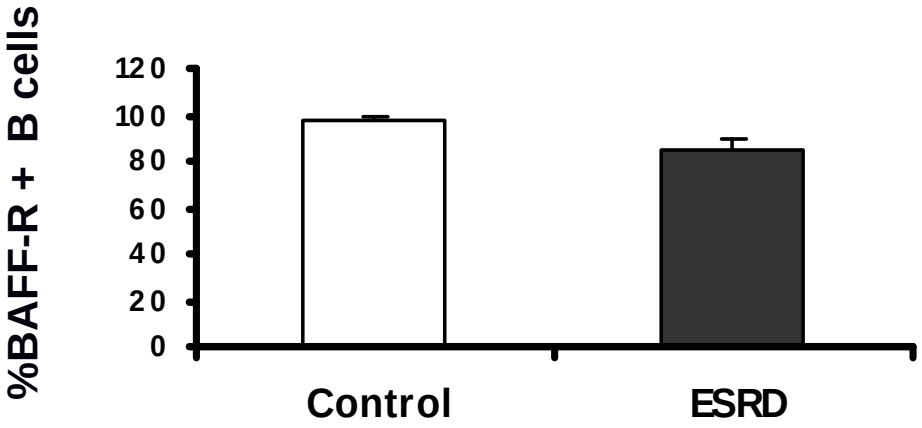
B cell apoptosis in ESRD

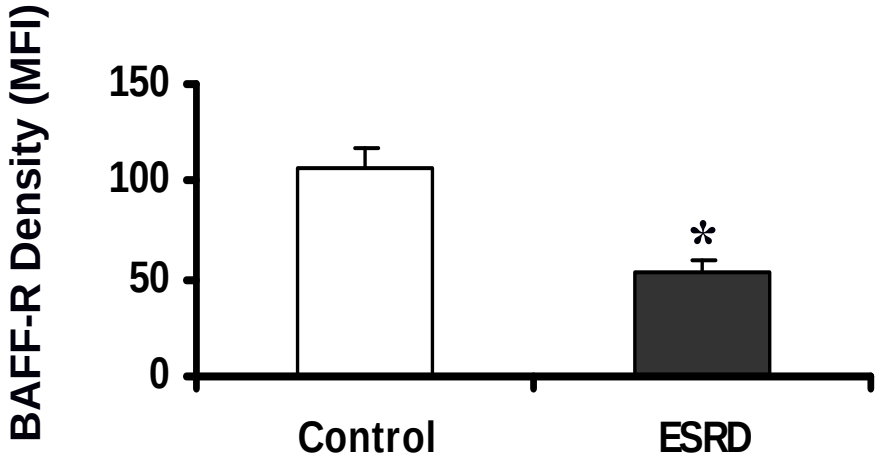
Plasma IL-7 and BAAF levels





BAAF receptor expression





Conclusions

- **The ESRD-associated inflammation is due to activation of innate immune system, orchestrated by monocytes, macrophages, granulocytes and cellular constituents of nearly all organs/tissues in the body**

- **The ESRD-associated inflammation is paradoxically coupled with immune deficiency which is caused by depletion of the antigen-presenting dendritic cells as well as naïve & central memory T cells and B cells and impaired phagocytic ability of monocytes and PMNs**

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