BMC Infectious Diseases

RESEARCH ARTICLE　　　　　　　　　　　　　　　　　　　　　　**Open Access**

Multicentric experience with interferon gamma therapy in sepsis induced immunosuppression. A case series

Didier Payen[1,2*] , Valerie Faivre[1,2], Jordi Miatello[3,4], Jenneke Leentjens[5], Caren Brumpt[6], Pierre Tissières[3,4], Claire Dupuis[1], Peter Pickkers[7†] and Anne Claire Lukaszewicz[1,2†]

Abstract

Background: The sepsis-induced immunodepression contributes to impaired clinical outcomes of various stress conditions. This syndrome is well documented and characterized by attenuated function of innate and adaptive immune cells. Several pharmacological interventions aimed to restore the immune response are emerging of which interferon-gamma (IFNγ) is one. It is of paramount relevance to obtain clinical information on optimal timing of the IFNγ-treatment, –tolerance, –effectiveness and outcome before performing a RCT. We describe the effects of IFNγ in a cohort of 18 adult and 2 pediatric sepsis patients.

Methods: In this open-label prospective multi-center case-series, IFNγ treatment was initiated in patients selected on clinical and immunological criteria early (< 4 days) or late (> 7 days) following the onset of sepsis. The data collected in 18 adults and 2 liver transplanted pediatric patients were: clinical scores, monocyte expression of HLA-DR (flow cytometry), lymphocyte immune-phenotyping (flow cytometry), IL-6 and IL-10 plasma levels (ELISA), bacterial cultures, disease severity, and mortality.

Results: In 15 out of 18 patients IFNγ treatment was associated with an increase of median HLA-DR expression from 2666 [IQ 1547; 4991] to 12,451 [IQ 4166; 19,707], while the absolute number of lymphocyte subpopulations were not affected, except for the decrease number of NK cells 94.5 [23; 136] to 32.5 [13; 90.8] (0.0625)]. Plasma levels of IL-6 464 [201–770] to 108 (89–140) ng/mL (p = 0.04) and IL-10 from IL-10 from 29 [12–59] to 9 [1–15] pg/mL decreased significantly. Three patients who received IFNγ early after ICU admission (<4 days) died. The other patients had a rapid clinical improvement assessed by the SOFA score and bacterial cultures that were repeatedly positive became negative. The 2 pediatric cases improved rapidly, but 1 died for hemorrhagic complication.

Conclusion: Guided by clinical and immunological monitoring, adjunctive immunotherapy with IFNγ appears well-tolerated in our cases and improves immune host defense in sepsis induced immuno suppression. Randomized clinical studies to assess its potential clinical benefit are warranted.

Keywords: Interferon gamma, Immuno-depression, MHC class II, Cytokines, Lymphocyte immuno-phenotyping, Sepsis

* Correspondence: dpayen1234@orange.fr
Peter Pickkers and Anne Claire Lukaszewicz are equally senior Authors.
[1]Groupe Hospitalier Saint-Louis Lariboisière, AP-HP, Université Paris 7 Denis Diderot, 2 rue Ambroise Paré, 75010 Paris, France
[2]UMR INSERM 1160 University Paris 7 Denis Diderot, Paris, France
Full list of author information is available at the end of the article

Background

One of the major advances in the field of sepsis during the last decade is the accumulation of evidence demonstrating the early shift in immune phenotype from an activated to a suppressed phenotype soon after the onset of sepsis [1]. Although the molecular mechanisms are not fully elucidated yet, the reported immune dysfunction of the cells from septic patients during immune-depression concern both the innate and adaptive immunity [2]. This phenotype is also observed in many other acute injuries suggesting a common pathophysiology after significant stress conditions as trauma [3], cardiopulmonary resuscitation [4], and major surgery [5]. However, if immunodepression is profound and persistent the patients become at risk of secondary infections [6] or are unable to resolve the primary infection [7].

To assess the patients' immune status, immune monitoring becomes essential. Until now, there are few adequate "immunoscope" candidates that are feasible in daily clinical practice and may facilitate the decision-making process. The quantification of the expression of the MHC class II molecule HLA-DR on circulating monocytes appears to represent a suitable parameter [8–11]. A persistent low level of HLA-DR expression is associated with the development of secondary infections and impaired clinical outcome [6, 12]. Of importance, it is becoming increasingly clear that the attenuated immune response can be reversed by different pharmacological interventions, including GM-CSF [13], IL-7 [14], anti-PD [15] and IFNγ [16]. Nowadays, as a last resort treatment, immunostimulating treatment is sometimes applied in critically ill sepsis patients and several small case series have been published [16]. Although effects on clinically relevant end-points can only be determined in randomized clinical trials, relevant data related to immunological properties, safety, and timing of treatment are needed to facilitate the design of such trials.

As previously reported [9], we choose the recombinant human IFNγ to treat immunodepression in different cases phenotype from 3 tertiary hospitals, including children in a context of liver transplantation. This molecule was selected for several reasons: 1- it is already therapeutically used for immune deficiency [17]; 2- the previous case reports did not mention severe side effects; and 3- the impact on innate immunity could be well monitored by the monocyte expression of HLA-DR. [10]

Methods

Patients and methods

Two different cohorts of septic patients (sepsis 2 definition) were collected. The *Cohort 1* of 13 adult patients having a sepsis-induced immunodepression syndrome was collected from May 2004 till 2017 in the Surgical

Intensive Care (SICU) at Lariboisière University Hospital, Paris, France from the large project "Severe Sepsis and inflammation monitoring" approved by Cochin Hospital Ethics Committee (# CCPPRB 2061), Assistance Publique Hôpitaux de Paris. The decision to administer IFNγ was made on the following criteria, which were not modified between 2004 and 2017: (1) an ICU stay over 7 days; (2) a diagnosis of secondary infection/colonization or an uncontrolled initial infection despite adequate antimicrobial therapy and/or interventional procedures; (3) a stable (at least 2 measurements) low level of mHLA-DR expression (<8000 antibody bound/cell (AB/C in our laboratory). Before IFNγ treatment (100mcg per subcutaneous injection, repeated at least 3 days with a maximum duration of 5 days) a written informed consent was obtained for each individual or from closest relative. The clear explanation of the potential risks and benefits to administer the drug as a compassionate treatment was applied according to the French Ethical law. For some patients, pro- and anti-inflammatory plasma cytokines levels were measured before and just after the end of IFNγ treatment. For the first time, the impact on lymphocyte immune phenotype was also evaluated.

The second *Cohort 2* had 4 patients from the Radboud University Medical Centre (Nijmegen, Netherlands). The patients were hospitalized for septic shock (Sepsis 2 definition) and were enrolled in a randomized clinical pilot trial (NCT 01649921). When norepinephrine infusion rate was reduced to 50% of maximum dose, ensuring that the sepsis-induced inflammation was declining (day 0), the administration of IFNγ (100mcg subcutaneous/day) was started. As a consequence, patients in this cohort could be treated with IFNγ earlier the day 7 in the ICU. This pilot trial was prematurely terminated due to a low enrollment rate.

In addition, 2 pediatric cases from the Pediatric Intensive Care (Kremlin-Bicêtre University Hospital) were added. Case 1: a 7 y/o transplanted the 1st time at the first year for fulminant hepatitis had to be transplanted again for chronic humoral rejection despite full treatment. She was referred for end-stage liver failure motivating an emergency liver transplantation 1 month after admission outlined by a hemorrhagic shock. After transplantation, continuous veno-venous hemodiafiltration was used for anuric renal failure and massive fluid overload. Under post-operative immunosuppression (basiliximab on day 1 and 4, methylprednisolone, tacrolimus and mycophenolate mofetil) an invasive aspergillosis (*Aspergillus fumigatus*) was confirmed in BAL, lumbar puncture and blood PCR with serial galactomannan antigenemia and blood PCR. Antifungal therapy associating voriconazole and caspofungine were initiated and immunosuppression stopped. The child developed

an acute and reversible hemorrhagic event related to multifocal intracerebral Aspergillosis lesions, and severe ARDS. In regard to the severity of the infection, rescue IFNγ was considered and proposed as a compassionate treatment. After parent's agreement and despite the modest reduction in mHLA-DR expression (mHLA-DR: 15,000 AB/C), one dose of 100 micrograms of IFNγ was subcutaneously injected. Although the improvement of the child condition (resolution of the respiratory failure and clinical awakening allowing to extubate), the child died on day 18 after transplantation with uncontrolled hemorrhage. Case 2: a 22-month-old boy originally transplanted for biliary atresia, was admitted to be transplanted again for recurrent cholangitis secondary to post-transplantation hepatic artery thrombosis and ischemic cholangitis.. The postoperative complications were: a renal tubulopathy; a delayed awaking and a suspicion of ventilatory associated pneumonia. He was rapidly re-intubated because of developed ARDS, associated with anuria, and septic shock with severe hypoxemia despite the use of all techniques to improve oxygenation. Distal lung protected sampling ($> 10^7$ cfu/ml) and ascites were positive for highly resistant *Pseudomonas aeruginosa*. Maximal supportive therapy associated with iv and aerosolized colistin with potential acquisition of resistance motivated discussion about IFNγ treatment. Monocytic HLA-DR measures were repetitively low (Day 11: mHLA-DR 2773 AB/C) (Fig. 2), 20 micrograms of IFNγ were injected subcutaneously during three consecutive days, with no significant side effect. The child dramatically improved hemodynamically at day 1 after the first IFNγ injection and was extubated 2 days later. Repetitive blood cultures and lung and abdominal samples remained negative. The child remains well thereafter. Both patients' immune monitoring of HLA-DR was monitored by the center A with the same protocol than cohort 1, using the same set up of flowcytometer.

IFNγ treatment

According to previous publication in human sepsis, a dose of 100mcg of IFNγ (Imukin®, Boehringer, Ingelheim, Germany) was subcutaneously injected for 3 to 5 days (cohort 1) or on days 2–4–7-9-11 (cohort 2) as reported [6, 9, 18, 19]. The treatment was repeated to reach an increased mHLA-DR expression above 8000 AB/C (cohort 1) or MFI > 20 in cohort 2 (low threshold fixed at MFI 20). Clinical tolerance and systemic or local (injection site) symptoms of inflammation were carefully checked.

Immune monitoring

The mHLA-DR expression was measured by flow cytometry (FACSCalibur and FACSCanto, Becton Dickinson, San Diego CA) as previously described in detail [6]. For the Lariboisière center (cohort 1), the median and IQ range of mHLA-DR expression in healthy people (*n* = 50) was 16, 884 [13,255–20,890] antibody bound per cell (AB/C). In cohort 2, mHLA-DR expression was assessed by the Mean Fluorescence Index (MFI, flow cytometry).

Plasma levels of IL-6 and IL-10 (ELISA method) were measured before, during and the day after the IFNγ treatment cessation in 6 patients of the cohort 1. The impact of IFNγ on lymphocyte subpopulations (CD3, CD4, CD8, CD19, and NK) was also investigated in 6 patients using classic immune-phenotyping method (see Additional files 1 and 2). The results were expressed in absolute values of subpopulations (central laboratory immune-phenotyping).

Because of the relatively small sample size, normal distribution could not be assumed and data is reported as median and interquartile range [IQR]. The non-parametric tests (Mann Whitney and Wilcoxon tests) were used appropriately to check the significant changes over time. Statistical SAS software, version 9.3 (SAS Institute Inc., Cary, North Carolina, USA) was used.

Results

Table 1 shows the characteristics of the cohort 1 and 2 of adult patients. In cohort 1, beside a slight increase in baseline body temperature (pre-treatment value 37.8 °C (37.5–38.4), the temperature increased to 39 °C (38.1–39.7), $p < 0.05$) during IFNγ treatment, no other side effects were observed. Figure 1a shows the individual mHLA-DR expression of the cohort 1 before and the day after stopping IFNγ stimulation with the delay from the ICU admission day. No relation between the time delay or the baseline level of mHLA-DR expression was observed for the drug response. All except one patient with a previously diagnosed Waldenström disease, increased their mHLA-DR expression. This non-responder patient to IFNγ died 3 days after the treatment initiation. Nine out of 13 survivors increased their mHLA-DR above the threshold of 8000 AB/C. In the remaining 4 patients, the HLA-DR expression increased, but not sufficiently to overpass the lower threshold limit and the treatment was stopped. Nevertheless, microbial cultures that remained positive prior to IFNγ treatment became negative in all patients. The bacterial cultures performed 6 days after the onset of IFNγ treatment (*i.e* 1 to 4 days after treatment) were all negative. The daily collected SOFA scores before during and after IFNγ administration decreased in 10 of 13 patients (Additional file 3: Figure S1). Only one patient having responded adequately to IFNγ treatment developed 6 days later a new pulmonary infection with positive cultures (*Pseudomonas aeruginosa*, Klebsiella and Aspergillus fumigatus) and recurrent immunodepression. This new infection episode occurred despite maintenance of adequate antimicrobial treatment concomitantly with a drop

Table 1 Clinical and infection characteristics of patients treated with IFNγ for cohort 1 and Cohort 2. AB/C = antibodies per cell. MFI = Mean Fluorescence Intensity

Cohort 1

	Age	Diagnosis at admission	Day of ICU	Secondary infection	microorganism	Antibiotic treatment	mHLA-DR (AB/C)	Injections of IFNγ	Day-15 outcome
1	30	Cardiac arrest	10	VAP	Pseudomonas aeruginosa	amikacin (4 days) + colimycin (2 days) + cefepim (2 days)	2419	5	alive
2	83	Postop cardiogenic shock	29	VAP	Pseudomonas aeruginosa, Stenotrophomonas maltophilia	ciprofloxacin (11 days) + ceftazidime (13 days)	4092	6	alive
3	73	Cardiogenic shock	16	VAP	Stenotrophomonas maltophilia, Pseudomonas aeruginosa	piperacillin + tazobactam (4 days)	1492	4	alive
4	63	Peritonitis	16	VAP	Pseudomonas aeruginosa	Imipenem (7 days)	1427	3	dead
5	42	Peritonitis	10	VAP, peritonitis	Pseudomonas aeruginosa, Aspergillus fumigatus	piperacillin+ tazobactam (10 days)	1547	6	alive
6	64	Peritonitis	37	Peritonitis VAP	Stenotrophomonas maltophilia + Enterobacter Cloacae + Enterococcus feacalis Enterobacter cloacae + Stenotrophomonas maltophilia	tigecycline + colimycin	2666	3	alive
7	65	Postoparative pneumonia	134	VAP	Streptococcus agalactiae	none	3289	5	alive
8	56	Pneumonia	11	VAP	Stenotrophomonas maltophilia EBV reactivation	piperacillin + tazobactam (10 days)	4991	4	alive
9	34	Pneumonia	15	VAP	Pseudomonas aeruginosa Aspergillus fumigatus	colimycine (15 days) amikacine (aerosolized) 15 days Voriconazole started	5428	5	alive
10	56	Cervical cellulitis septicemia	13	Perirenal abscess	Staphylococcus aureus	oxacilline + Pefloxacine (12 days)	2056	7	alive
11	60	Fasciitis	38	Fasciitis	Pseudomonas aeruginosa	Imipenem + amikacine (3 days)	5132	5	alive
12	74	Keto-acidosis	40	VAP, lung abcess, pleuresis	Pseudomonas aeruginosa, Citrobacter freundii	Imipenem (7 days)	7073	4	Alive
13	82	Rectal Fistulae & fasciitis	9	Tight muscle infection	Gram negative multiple bacteria anaerobes	Piperacillin + tazobactam (4 days) Imipenem + amikacine (7 days)	2168	3	Alive

Cohort 2

Age	Diagnosis at admission	Infection focus	Delay between admission and IFNγ	Microorganism	Antibiotic treatment	SOFA-score at admission	mHLA-DR Expression (MFI)	Outcome
74	Septic Shock	Abdominal	4	Unknown	Ceftriaxon	12	16.0	Death
73	"	Abdominal	3	Klebsiella pneumoniae	Ceftriaxon	8	14.6	Alive
74	"	Bilary	1	Multi-resistant E coli, Clostridium perfringens	Piperacillin / tazobactam, ceftriaxon erytromycin	14	5.6	Alive
80	"	Abdominal	5	Staphylococcus haemolyticus, Candida albicans	Vancomycin and Piperacillin / tazobactam, myfungin / fluconazole	9	73.6	Death

VAP Ventilator-associated pneumonia, *EBV* Ebstein Barr virus, *CMV* cytomegalovirus

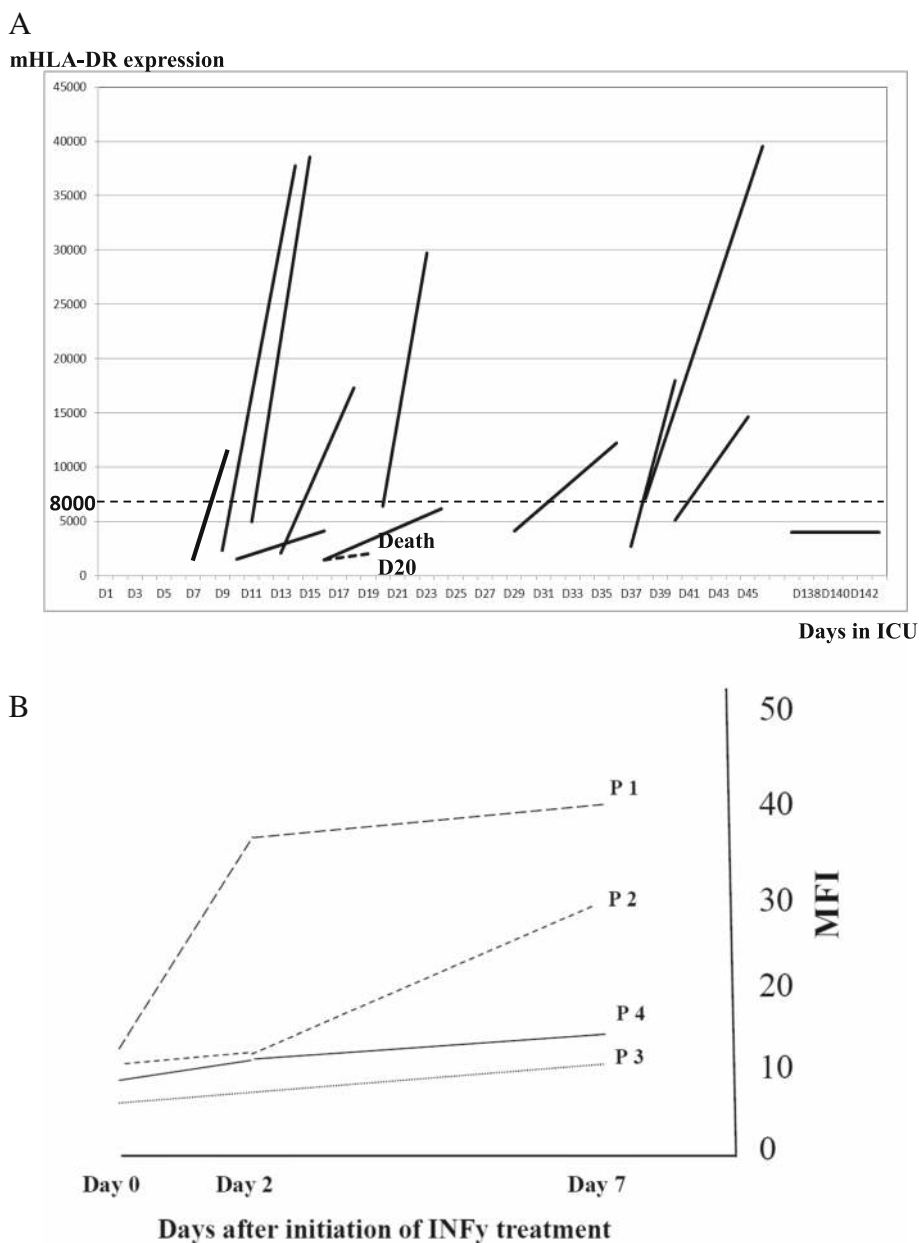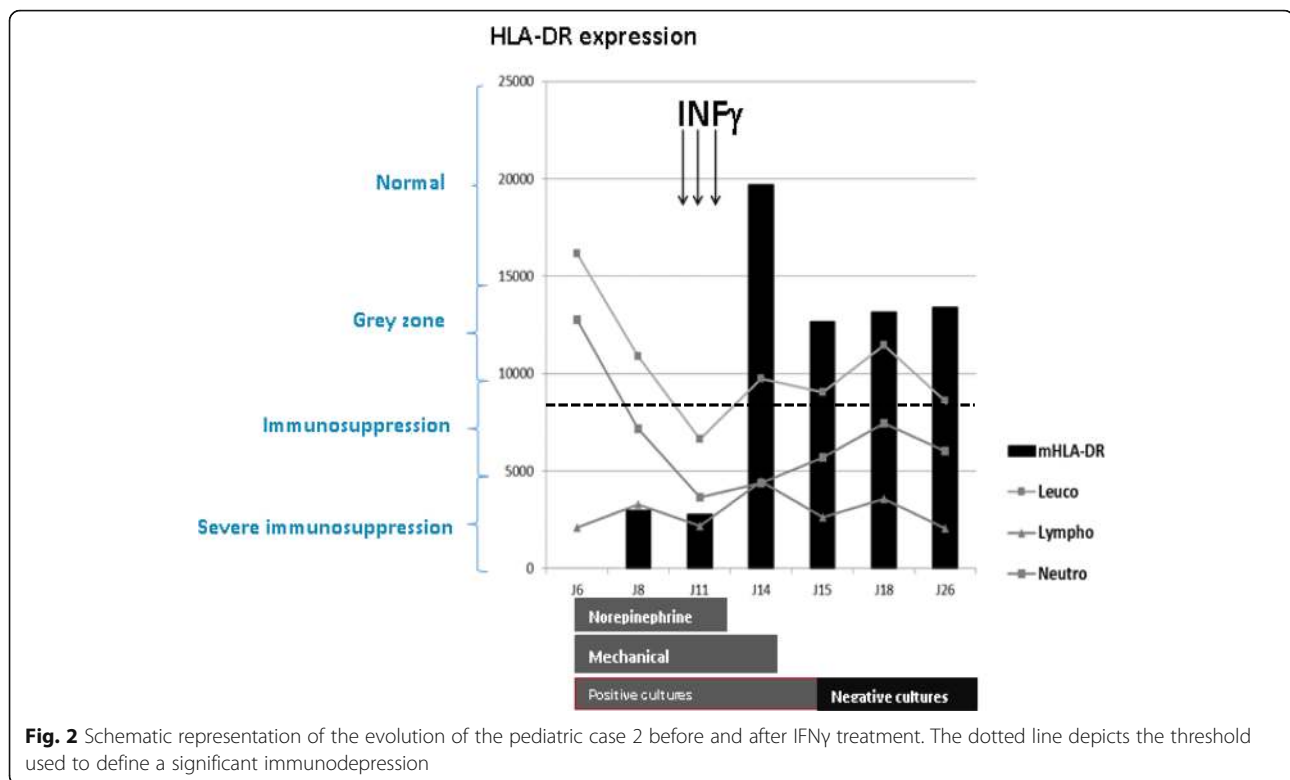Payen *et al. BMC Infectious Diseases* (2019) 19:931

Page 5 of 10

Fig. 1 shows the evolution of monocyte HLA-DR expression. Figure 1**a** (cohort A) depicts the individual data of mHLA-DR expression (AB/C event/cell) before and within the 24 h after stopping IFNγ treatment, showing the real delay from the admission to be treated. The dotted line figures the threshold below which the immunodepression is identified. Among the 13 patients, 4 increased the HLA-DR expression but did not reach the defined threshold. The X axis: days from admission; the Y axis represents the quantitative AB/C values of mHLA-DR expression. Figure 1**b** (cohort B) shows similar representation: the X axis: days were IFNγ treatment was administered; Y axis represents the MFI level

in mHLA-DR expression (Additional file 4: Figure S2). The medical team decided to restart the IFNγ treatment for 4 days inducing again the resolved infection accompanied by a quick improvement in clinic and in chest CT scan (Additional file 4: Figure S2). After discharge from ICU, 4 patients died at day 52, 86, 108 and day 176 post admission.

In cohort 2, in accordance with the protocol (NCT 01649921) the 1st dose of IFNγ was administered between day 1 and 4 after admission to ICU for septic shock. Table 1 summarizes the baseline characteristics and outcomes. The 2 cohorts were different for several items: the cohort 2 was older with a higher APACHE II score level and dose of norepinephrine infusion at time

Payen *et al. BMC Infectious Diseases* (2019) 19:931

Page 6 of 10

Fig. 2 Schematic representation of the evolution of the pediatric case 2 before and after IFNγ treatment. The dotted line depicts the threshold used to define a significant immunodepression

of decision to use IFNγ. Figure 2b depicts the individual evolution of mHLA-DR levels expressed in MFI of variations from pre-treatment values. Initial mHLA-DR levels were low (below MFI 20) in 3 out of 4 patients and increased during IFNγ treatment. In one patient the IFNγ-induced a rapid increase in mHLA-DR expression, promptly followed by a sharp decrease of HLA-DR expression, associated with clinical deterioration and death. Two patients died within the 14-days after initiation of IFNγ treatment. No serious adverse events linked to IFNγ treatment were observed.

Pediatric cases

Both had uncontrolled infection after liver transplantation despite the full supportive and adequate microbial therapies. The expression of mHLA-DR demonstrated a sharp increase when IFNγ was administered. The usual immunosuppressive drugs to prevent graft rejection after liver transplantation were maintained. Figure 2 illustrates the evolution of mHLA-DR expression in the case n°2 before, during, and after administration of 20 mcg/subcutaneous injection of IFNγ for 5 days.
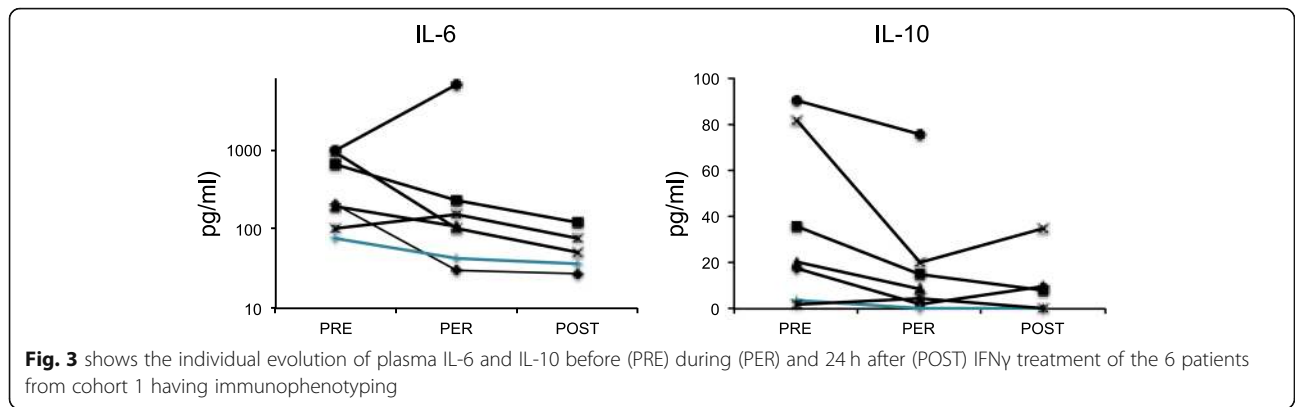
Subgroup for immunological evaluation

Figure 3 summarizes the course of individual plasma IL-6 and IL-10 levels from baseline to the day post IFNγ treatment (n = 6 from cohort 1). IL-6 decreased from 464 [201–770] to 108 (89–140) ng/mL (p = 0.04) with a

decrease in IL-10 from 29 [12–59] to 9 [1-15] pg/mL (p = 0.06). Figure 4 shows the individual evolution of the patients from the cohort 1 having lymphocyte immune-phenotyping. Treatment with IFNγ did not modify the %, nor the absolute value of lymphocyte subpopulations, except for NK cells. IFNγ treatment induced a clear trend of decreased absolute value of NK cell subtype from 13 [12-14] to 10 [3-10] (p = 0.0625). Figure 5 is a schematic proposal to consider a potential treatment for acute severe immunodepression that could be applied for future randomized control trials.
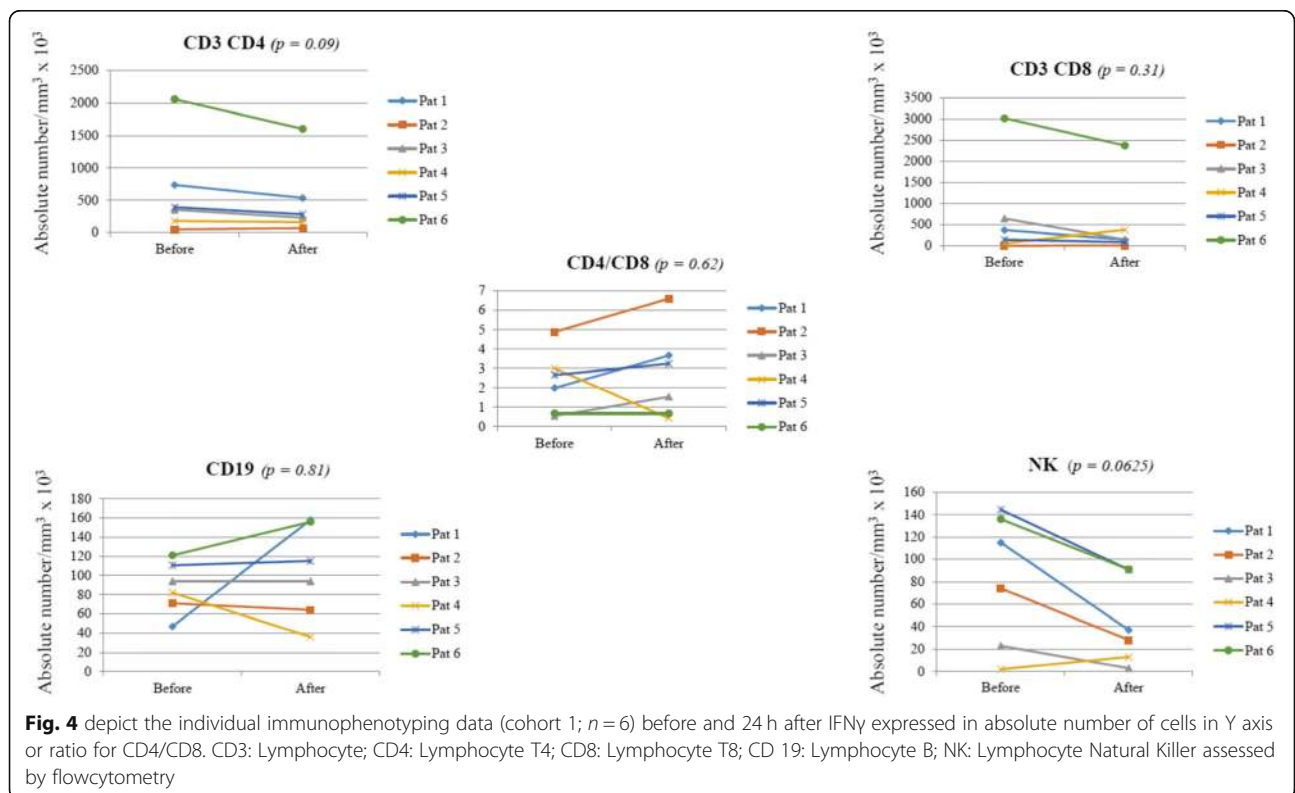
Discussion

Over the last decades it is increasingly recognized that a large proportion of sepsis patients suffer from a sustained suppression of their immune system [1]. This immune-suppression is associated with reactivation of latent viral infections [20], the development of secondary infections and impaired clinical outcomes [6, 21]. Several pharmacological interventions may restore the immune response, but adequately large clinical trials are currently lacking. We investigated the safety and immunological effects of IFNγ in 17 adult patients reported that IFNγ increased monocytic HLA-DR expression in all except one patient. Treatment with IFNγ did not boost circulating cytokine concentrations: IL-6 and IL-10 concentrations were lower than pretreatment values. It tended to decrease NK-cell proportion without changes for other
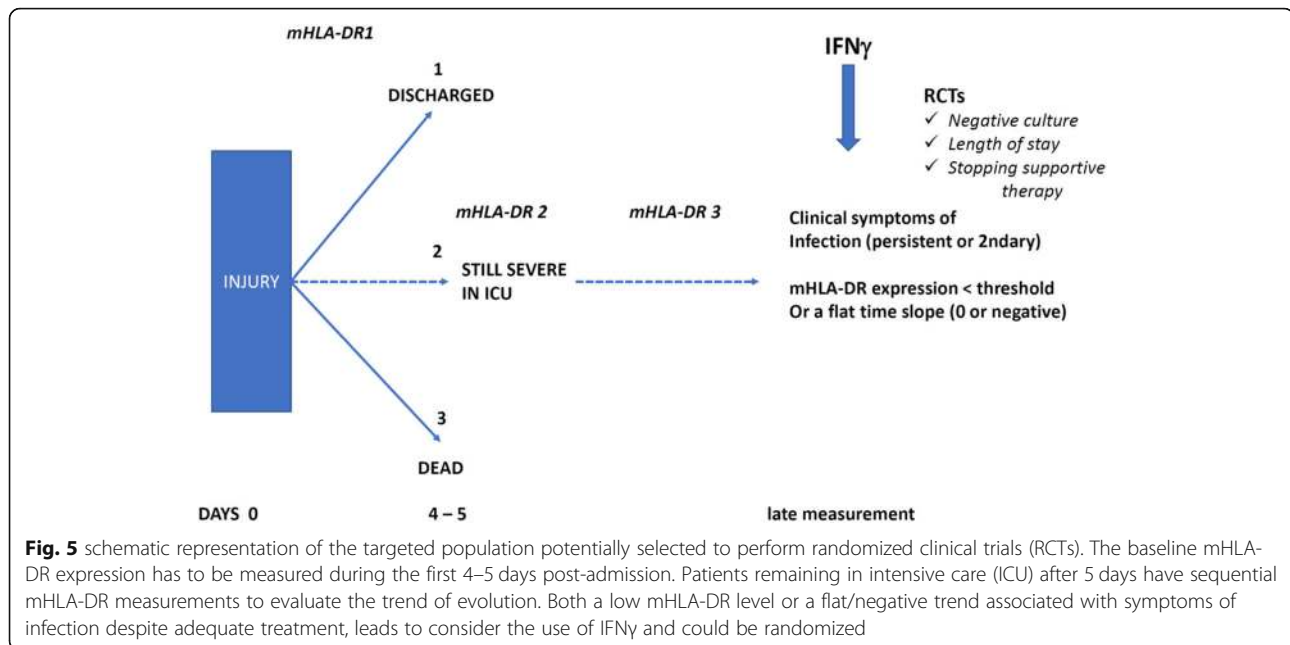
Fig. 3 shows the individual evolution of plasma IL-6 and IL-10 before (PRE) during (PER) and 24 h after (POST) IFNγ treatment of the 6 patients from cohort 1 having immunophenotyping

leukocyte subpopulations. In addition, cultures that remained positive prior to IFNγ treatment became negative in all patients. No safety effects judged to be attributable to IFNγ were observed. Overall, survival appeared higher than anticipated in this specific group of immunoparalyzed patients, but clearly the numbers are too small to conclude that the restoration of the immune response is related to better clinical outcome.

The IFNγ used in the present case series was a human type II IFNγ having the advantage to be 100–1000 more active than other interferons [22]. This molecule received the FDA approval for clinical application against chronic granulomatous disease to decrease the severity and number of infections. Beneficial effects of adjuvant

treatment using IFNγ for fungal infections in patients suffering from chronic granulomatous disease [17] HIV [23–25], leukemia [26, 27], and in organ transplant patients [28] were previously reported. The first report using this IFNγ in human bacterial sepsis was made by Docke et al. [9] in 9 septic ICU patients at 100mcg daily dose. Since then, several small case series suffering from opportunistic infections [16] [29] or pediatric case [30, 31] or HIV cases [23–25] have been published. The present case series is the first reporting the use of IFNγ in adult and pediatric bacterial sepsis, including septic shock patients with associated cytokines level modifications and immunophenotyping. The delay to use IFNγ from the ICU hospitalization differed largely within the

Fig. 4 depict the individual immunophenotyping data (cohort 1; n = 6) before and 24 h after IFNγ expressed in absolute number of cells in Y axis or ratio for CD4/CD8. CD3: Lymphocyte; CD4: Lymphocyte T4; CD8: Lymphocyte T8; CD 19: Lymphocyte B; NK: Lymphocyte Natural Killer assessed by flowcytometry

Fig. 5 schematic representation of the targeted population potentially selected to perform randomized clinical trials (RCTs). The baseline mHLA-DR expression has to be measured during the first 4–5 days post-admission. Patients remaining in intensive care (ICU) after 5 days have sequential mHLA-DR measurements to evaluate the trend of evolution. Both a low mHLA-DR level or a flat/negative trend associated with symptoms of infection despite adequate treatment, leads to consider the use of IFNγ and could be randomized

2 reported small cohorts. One might argue that the un-favorable outcome in 2 patients of cohort 2 early treated may suggest that IFNγ therapy should not be used too early, even when HLA-DR expression is already sup-pressed. In the early treated group, the patients are still in the steep part of the survival curve with a high mor-tality (close to 50%) of severe septic shock patients [32]. Nevertheless, the cases of the cohort 2 early treated (< 4 days after admission for septic shock) had a rapid down-regulation of HLA-DR expression that responded well to IFNγ stimulation for mHLA-DR expression. The lack of positive signal for outcome could be related to a down-regulation adapted to the acute phase with no benefit to administer IFNγ.

The concentrations of circulating cytokines were mea-sured in cohort 1 to examine whether or not the immu-nostimulating with IFNγ would result in increases in these cytokines, which could have deleterious effects. This was not the case, both circulating IL-6 and IL-10 levels decreased following IFNγ treatment. In accordance, in volunteers exposed to endotoxin twice, treatment with IFNγ restored the suppressed cytokine response during the second endotoxin exposure. So, IFNγ prevents and reverses the in vivo induced endotoxin tolerance with no indications of an overshoot response [18]. The modest changes induced by IFNγ in lymphocytes subpopulations were observed, except for absolute number of NK cells. The clear trend of NK cell decrease in our case series may result from a migration into infected tissue or from a reduction in NK cell generation. Being poorly reported, this observation made in a small size cohort should be cautiously interpreted. The reported case series on

invasive fungi infection did not show changes in the total leucocyte and granulocyte numbers when IFNγ-treatment was given change [16].

The predominant effect on innate immunity associated with the moderate changes of adaptive immunity, opens the possibility to use IFNγ even after organ transplant-ation maintaining the anti-rejection treatment targeting mainly the lymphocytes. It is possible and reasonable to combine the IFNγ treatment with the maintenance of an anti-graft rejection treatment when an infection cannot be controlled.

The clinical benefit of IFNγ treatment appeared favor-able in cohort 1 with only 1 death. All cohort except 1 patient improved enough to be discharged from ICU. This observation suggests that IFNγ is most clinically ef-fective if the suppressed immune response is present for a longer period and is associated with new infections. We hypothesized that the duration more than the depth of mHLA-DR downregulation is relevant for the clin-ician to detect the risk of new infections. This aspect should be verified in a larger population. As reported previously, the IFNγ treatment was well tolerated in our 20 patients with no clearly attributable severe side ef-fects. The monitoring of HLA-DR expression on circu-lating monocytes appears adequate to follow the immunological efficacy of the compound, a relatively cheap immune monitoring exam to facilitate the clinical decision making.

This case series has important limitations, mostly re-lated to its observational nature and the compassionate use of the drug. First, the 20 collected cases are too limited to draw definitive conclusions about the clinical

benefit of this treatment. However, grouping together the case reports and the other case series results in at least 50 reported cases treated by IFNγ. Overall, an excellent tolerance was reported using a 100 mcg daily in adult. It is still essential to determine the dose-response relationship, and the tolerance of potential repetitive treatment to complete the safety profile of the compound. Despite the small number of cases, it is remarkable that mHLA-DR significantly increased following the first injection and went down again rapidly after treatment cessation. Second, macrophage or monocyte polarization (M1 or M2) has not been tested in our cases, which hampered the understanding of re-programming mechanisms and its impact on monocyte-macrophage polarization.

Conclusion

Sepsis induces suppression of the immune system, associated with susceptibility to secondary infections and impaired clinical outcome. We report cases for whom IFNγ treatment was well-tolerated and improved immune host defense. The increase in monocytic HLA-DR expression did not induce a storm of cytokine release nor a modification in lymphocyte immunophenotyping, except for decrease in absolute number of NK cells. Within the limits of small size cohort, the clinical benefit of IFNγ to stimulate innate immunity in presence of immunosuppression is an attractive track for the future. The Fig. 5 illustrates the potential design for future clinical trials. The primary end-point might be the resolution of infection and/or positive culture and the length of stay in intensive care.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12879-019-4526-x.

Additional file 1. Supplementary methods

Additional file 2: Supplementary pediatric cases

Additional file 3: Figure S1. Variations in SOFA score before and after IFNγ treatment in cohort A. Legend: Vertical axis: % of SOFA variations; horizontal axis: days before and after treatment by INFγ.

Additional file 4: Figure S2. Evolution of pulmonary CT scan over days in patient 8.

Abbreviations

CD: Cluster of Differentiation; HIV: Human Immunodeficiency Virus; HLA-DR: Human Leucocyte Antigen-DR; ICU: Intensive Care Unit; IFNγ: interferon gamma; IL-10: interleukine 10; IL-6: interleukine 6; MFI: Mean Fluorescence intensity; MHC class II: Major Histocompatibility Complex II; NK: Natural Killer; PCR: Polymerase Chain Reaction; PD: Program Death; RCT: Randomized Clinical Trial; SICU: Surgical Intensive Care Unit; SOFA: Sequential Organ Failure Assessment

Acknowledgements

The authors thank the medical team of concerned ICUs, especially Doctor Matthieu Le Dorze.

Authors' contributions

DP, PT and ACL and PP conceived and designed the data collection. DP, ACL, JM, PT, JL, and PP screened and included patients. VF, CB and JL carried out flow cytometry, immunophenotyping, and cytokine measurements. DP, PP, and CD analyzed the data. DP, PP, ACL, JL, PT and JM participated in the data interpretation. DP, ACL, PT and PP wrote the manuscript draft. DP, ACL, PP, CB and FV contributed to the writing of the final manuscript. All authors read and approved the final manuscript

Availability of data and materials

According to the ethical medical protection in France, the case data cannot be diffused. The data can be obtained on specific request to the authors.

Ethics approval and consent to participate

The cohort 1 was collected from the data of the large project on "Severe Sepsis and inflammation monitoring" approved by Cochin Hospital Ethics Committee (# CCPPRB 2061), Assistance Publique-Hôpitaux de Paris. For each patient of the cohort 1, a clear explanation of the justification to use IFNγ was given, based on the literature and on the experience of the research clinical team. According to the French law and with the agreement of the Institutional Pharmacy Board, IFNγ was proposed as a compassionate treatment with clear explanation given to the patient's next of kin about the potential risks and benefits.
For each pediatric case, IFNγ administration was a multidisciplinary decision between intensivists, surgeons and hepatologists, which was proposed to the parents as a compassionate treatment. The compassionate therapy by IFNγ was validated by institutional pharmacist after a review of the literature. Decisional process was notified in the hospitalization records and synthesis note with signature on the medical charts.

Consent for publication

Written informed consent was obtained from the parent of the patients for publication of these Case Report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

Competing interests

None of the authors have any competing interests regarding this study. In this study, the support for the immunological assessments was covered by payment of additional immune tests in the global cost for care.

Author details

[1]Groupe Hospitalier Saint-Louis Lariboisière, AP-HP, Université Paris 7 Denis Diderot, 2 rue Ambroise Paré, 75010 Paris, France. [2]UMR INSERM 1160 University Paris 7 Denis Diderot, Paris, France. [3]Pediatric Intensive Care and Neonatal Medicine, Bicêtre Hospital, AP-HP, Le Kremlin-Bicêtre, France. [4]Institute of Integrative Biology of Cell, CNRS, CEA, Univ. Paris Sud, Paris Saclay University, Gif sur Yvette, France. [5]Departments of intensive care and internal medicine, Radboud university medical center Nijmegen, PO box 9101, 6500 HB Nijmegen, The Netherlands. [6]Service d'Hématologie Biologique, Pôle B2P, Hôpital Lariboisière, APHP, Paris, France. [7]Department Intensive Care Medicine, Radboud university medical center Nijmegen, PO box 9101, 6500 HB Nijmegen, The Netherlands.

Payen *et al. BMC Infectious Diseases*   (2019) 19:931

Page 10 of 10

References

1. Hotchkiss RS, Monneret G, Payen D. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. Lancet Infect Dis. 2013;13(3):260–8.
2. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. Nat Rev Immunol. 2013; 13(12):862–74.
3. Gouel-Cheron A, Allaouchiche B, Floccard B, Rimmele T, Monneret G. Early daily mHLA-DR monitoring predicts forthcoming sepsis in severe trauma patients. Intensive Care Med. 2015;41(12):2229–30.
4. Venet F, Cour M, Demaret J, Monneret G, Argaud L. Decreased monocyte HLA-DR expression in patients after non-Shockable out-of-hospital cardiac arrest. Shock. 2016;46(1):33–6.
5. Klava A, Windsor A, Boylston AW, Reynolds JV, Ramsden CW, Guillou PJ. Monocyte activation after open and laparoscopic surgery. Br J Surg. 1997; 84(8):1152–6.
6. Lukaszewicz AC, Grienay M, Resche-Rigon M, Pirracchio R, Faivre V, Boval B, Payen D. Monocytic HLA-DR expression in intensive care patients: interest for prognosis and secondary infection prediction. Crit Care Med. 2009; 37(10):2746–52.
7. Boomer JS, To K, Chang KC, Takasu O, Osborne DF, Walton AH, Bricker TL, Jarman SD 2nd, Kreisel D, Krupnick AS, et al. Immunosuppression in patients who die of sepsis and multiple organ failure. JAMA. 2011;306(23):2594–605.
8. Docke WD, Hoflich C, Davis KA, Rottgers K, Meisel C, Kiefer P, Weber SU, Hedwig-Geissing M, Kreuzfelder E, Tschentscher P, et al. Monitoring temporary immunodepression by flow cytometric measurement of monocytic HLA-DR expression: a multicenter standardized study. Clin Chem. 2005;51(12):2341–7.
9. Docke WD, Randow F, Syrbe U, Krausch D, Asadullah K, Reinke P, Volk HD, Kox W. Monocyte deactivation in septic patients: restoration by IFN-gamma treatment. Nat Med. 1997;3(6):678–81.
10. Venet F, Lukaszewicz AC, Payen D, Hotchkiss R, Monneret G. Monitoring the immune response in sepsis: a rational approach to administration of immunoadjuvant therapies. Curr Opin Immunol. 2013;25(4):477–83.
11. Monneret G, Venet F, Meisel C, Schefold JC. Assessment of monocytic HLA-DR expression in ICU patients: analytical issues for multicentric flow cytometry studies. Crit Care. 2010;14(4):432.
12. Pickkers P. Simultaneously mounted pro- and anti-inflammatory host response relates to the development of secondary infections in patients with Sepsis. Am J Respir Crit Care Med. 2017;196(4):406–7.
13. Meisel C, Schefold JC, Pschowski R, Baumann T, Hetzger K, Gregor J, Weber-Carstens S, Hasper D, Keh D, Zuckermann H, et al. Granulocyte-macrophage colony-stimulating factor to reverse sepsis-associated immunosuppression: a double-blind, randomized, placebo-controlled multicenter trial. Am J Respir Crit Care Med. 2009;180(7):640–8.
14. Francois B, Jeannet R, Daix T, Walton AH, Shotwell MS, Unsinger J, Monneret G, Rimmele T, Blood T, Morre M, et al. Interleukin-7 restores lymphocytes in septic shock: the IRIS-7 randomized clinical trial. JCI Insight. 2018;3:5.
15. Hotchkiss R, Olston E, Yende S, Angus D, Moldawer L, Crouser E, Martin G, Coopersmith C, Brakenridge S, Mayr F, et al. Immune checkpoint inhibition in Sepsis: a phase 1b randomized, placebo-controlled, single ascending dose study of Antiprogrammed cell death-ligand 1 antibody (BMS-936559). Crit Care Med. 2019;47(5):632–42.
16. Delsing CE, Gresnigt MS, Leentjens J, Preijers F, Frager FA, Kox M, Monneret G, Venet F, Bleeker-Rovers CP, van de Veerdonk FL, et al. Interferon-gamma as adjunctive immunotherapy for invasive fungal infections: a case series. BMC Infect Dis. 2014;14:166.
17. A controlled trial of interferon gamma to prevent infection in chronic granulomatous disease. The International Chronic Granulomatous Disease Cooperative Study Group. N Engl J Med 1991, 324(8):509–516.
18. Leentjens J, Kox M, Koch RM, Preijers F, Joosten LA, van der Hoeven JG, Netea MG, Pickkers P. Reversal of immunoparalysis in humans in vivo: a double-blind, placebo-controlled, randomized pilot study. Am J Respir Crit Care Med. 2012;186(9):838–45.
19. Payen DM, Guilhot J, Launey Y, Lukaszewicz AC, Kaaki M, Veber B, Pottecher J, Joannes-Boyau O, Martin-Lefevre L, Jabaudon M, et al. Early use of polymyxin B hemoperfusion in patients with septic shock due to peritonitis: a multicenter randomized control trial. Intensive Care Med. 2015;41(6):975–84.
20. Kaufmann SHE, Dorhoi A, Hotchkiss RS, Bartenschlager R. Host-directed therapies for bacterial and viral infections. Nat Rev Drug Discov. 2018;17(1): 35–56.
21. Landelle C, Lepape A, Voirin N, Tognet E, Venet F, Bohe J, Vanhems P, Monneret G. Low monocyte human leukocyte antigen-DR is independently associated with nosocomial infections after septic shock. Intensive Care Med. 2010;36(11):1859–66.
22. Razaghi A, Owens L, Heimann K. Review of the recombinant human interferon gamma as an immunotherapeutic: impacts of production platforms and glycosylation. J Biotechnol. 2016;240:48–60.
23. Bodasing N, Seaton RA, Shankland GS, Pithie A. Gamma-interferon treatment for resistant oropharyngeal candidiasis in an HIV-positive patient. J Antimicrob Chemother. 2002;50(5):765–6.
24. Jarvis JN, Meintjes G, Rebe K, Williams GN, Bicanic T, Williams A, Schutz C, Bekker LG, Wood R, Harrison TS. Adjunctive interferon-gamma immunotherapy for the treatment of HIV-associated cryptococcal meningitis: a randomized controlled trial. AIDS. 2012;26(9):1105–13.
25. Riddell LA, Pinching AJ, Hill S, Ng TT, Arbe E, Lapham GP, Ash S, Hillman R, Tchamouroff S, Denning DW, et al. A phase III study of recombinant human interferon gamma to prevent opportunistic infections in advanced HIV disease. AIDS Res Hum Retrovir. 2001;17(9):789–97.
26. Dignani MC, Rex JH, Chan KW, Dow G, de Magalhaes-Silverman M, Maddox A, Walsh T, Anaissie E. Immunomodulation with interferon-gamma and colony-stimulating factors for refractory fungal infections in patients with leukemia. Cancer. 2005;104(1):199–204.
27. Poynton CH, Barnes RA, Rees J. Interferon gamma and granulocyte-macrophage colony-stimulating factor for the treatment of hepatosplenic candidosis in patients with acute leukemia. Clin Infect Dis. 1998;26(1):239–40.
28. Armstrong-James D, Teo IA, Shrivastava S, Petrou MA, Taube D, Dorling A, Shaunak S. Exogenous interferon-gamma immunotherapy for invasive fungal infections in kidney transplant patients. Am J Transplant. 2010;10(8): 1796–803.
29. Nakos G, Malamou-Mitsi VD, Lachana A, Karassavoglou A, Kitsiouli E, Agnandi N, Lekka ME. Immunoparalysis in patients with severe trauma and the effect of inhaled interferon-gamma. Crit Care Med. 2002;30(7):1488–94.
30. Leentjens J, Gresnigt MS, van de Veerdonk FL, Kox M, Kullberg BJ, Pickkers P, Brouwer AE, Netea MG. Adjuvant interferon-gamma immunotherapy in a patient with progressive cerebral Nocardia abscesses. Int J Infect Dis. 2017; 59:25–8.
31. Tissieres P, Ochoda A, Dunn-Siegrist I, Drifte G, Morales M, Pfister R, Berner M, Pugin J. Innate immune deficiency of extremely premature neonates can be reversed by interferon-gamma. PLoS One. 2012;7(3):e32863.
32. Sweeney TE, Wong HR. Risk stratification and prognosis in Sepsis: what have we learned from microarrays? Clin Chest Med. 2016;37(2):209–18.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.