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Viral transformation for production of personalized type I interferons

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Abstract

Type I interferons (IFN) are cytokines with many functions and have been widely used to treat many human diseases such as hepatitis C virus (HCV) infection. By the use of viral transformation and priming properties of Epstein-Barr virus (EBV), we have developed a system that can produce high levels of “personalized” IFNs, which are produced from the cells of the patient to whom the IFNs are to be administered. We demonstrate the feasibility of the system. This seems to be the first report for the establishment of a personalized IFN production system. The personalized IFNs could have longer circulation time, fewer side-effects but higher efficacy. We anticipate that the system can provide an improved form of IFN for medical uses.

Keywords

Red Biotechnology; Interferon; type I IFNs; personalized medicine; cytokine

Type I interferons (IFNs) are cytokines with many functions including antiviral and anti-proliferation [1,2]. IFNs contain 13 IFN- α , 1 IFN- β , and 1 IFN- ω genes, and are widely used in therapeutic approaches to treat hepatitis C virus (HCV) infection, to stop the growth and spread of cancer cells (*e.g.*, melanoma), and to treat other diseases of viral, malignant, angiogenic, allergic, inflammatory, and fibrotic origin. IFNs for medical use are mainly produced using recombinant DNA technology with one or few IFN subtypes. Natural IFNs are also used, which have multiple subtypes produced from cultured human cells or primary peripheral blood leukocytes (PBMC) isolated from blood.

IFNs have been characterized by a number of side effects from flu-like symptoms to organ failure. In addition, current IFN treatments have a relatively low responding rate. Because of side effects and dismal responding rate, it is estimated that only 5% of U.S. hepatitis C patients are being treated. When safer and more effective breakthrough HCV therapies reach the market, the number of patients treated is expected to expand substantially. Thus, there remains a significant need for an improved IFN that can be characterized by minimal side effects and high efficacy in patients undergoing IFN therapy.

Epstein-Barr virus (EBV) is a ubiquitous human gamma herpesvirus. EBV infects and converts adult primary B cells into continually growing lymphoblastoid cell lines (LCLs) *in vitro* [3-5]. EBV latent membrane protein 1 (LMP-1) is required for EBV transformation of primary B cells. Interestingly, LMP-1 is an antiviral gene and primes EBV latency cells for

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type I IFN productions [6,7]. The LMP-1-mediated priming is at least partially related to its ability to induce IFN regulatory factor 7 (IRF-7) [8-10]. IRF-7 is the master activator for IFN expression [11,12].

We have designed a system for the generation of personalized IFNs (Figure 1). This system is basically harnessing the two special properties of EBV: 1) the ability to transform primary B lymphocytes; and 2) the ability to prime cells for robust IFN productions [6].

To test the achievability of personalized IFNs (Figure 1), blood from donors was used to isolate PBMCs, and subsequently infected with EBV. The proliferating cells were collected and expanded. When more than 10 ml of the cell cultures was obtained, Sendai virus was used to infect these cells. Both IFN- α and IFN- β were highly induced (Figure 2A, 2B). Thus, the designed system can produce personalized type I IFNs in a small scale, at least in Steps 1, 2, as well as Step 3 before the purification (Figure 1).

Medical used natural IFNs can be generated from Namalwa cells or fresh isolated PBMCs. IB4 is a prototypical EBV-transformed primary B cell line *in vitro*. Cells were infected by same amounts of Sendai viruses from the same batch. As shown in Figure 2C, comparable amounts of IFNs can be produced among IB4, Namalwa, as well as fresh PBMCs, which suggest that manufacturing potential of EBV-transformed cells as IFN production sources may be as good as other commercially available cell sources. The IB4 cell line was established many years ago [13] and had been maintained in our laboratory for more than two year. Thus, long-term-cultured EBV transformed cells are capable of producing robust levels of IFNs.

IFN is the major treatment for hepatitis C patients, melanoma and other disease. It is known that both HCV and melanoma patients' B cells are able to be transformed by EBV [14,15]. In addition, EBV LMP-1 is required for viral transformation and IRF-7, a master gene for IFN production, is associated with EBV transformation and induced by LMP-1, it is thus likely that EBV transformed cells are able to produce IFNs.

Antibodies to administered IFNs would inhibit and even neutralize the activities of the IFNs [16,17]. Because personalized ones are from individual's own cells, the conformation of the proteins is likely to be very similar to its counterparts *in vivo*. Therefore the personalized ones would greatly reduce the likelihood of antibody responses to the IFNs administered to the specific individual. The circulation time of the functional IFNs in patients' body is expected to be longer than conventional IFNs. The longer circulation time may enhance efficiency and reduce side effects.

IFNs are used to treat diseases in adults. The specific patient probably already encounters the IFNs with the subtype combination/glycosylation patterns many times in his/her life time. Therefore, the personalized IFNs are likely to be the most adaptable ones to the specific person. Although the major side effects of IFNs are mechanism based, however, other side-effect inducing mechanism, such as those related to the IFN sequence variants or alleles [18,19], or conformational variations as well as those arising from chronic use, may be minimized.

Type I IFNs have many subtypes including IFN- α and β . Removal of one single subtype (IFN- β) in mouse has lead to reduced innate immunity [20]. In addition, different IFN- α subtypes have distinct pattern of antiviral specific activity on different cells [21]. Thus, each subtype and/or the combination of several subtypes may have a special function/effect *in vivo*. Some recombinant IFN-resistant hairy cell leukemia patients, *i.e.*, resistant to one subtype of IFNs, were treated effectively with natural IFNs (multiple IFNs derived from human cells) [22]. Also, some IFN subtypes are glycosylated, glycosylation may affect

biological activities. Personalized IFNs would have multiple subtypes and may have identical post-translational modifications as in the specific individuals. Therefore, personalized IFNs may have higher efficiency than the majority of the currently used IFNs, which are predominantly one or fewer subtypes.

The safety issues of the personalized IFNs would be similar to other natural IFNs. IFNs derived from Namalwa (EBV-positive) cells by infection with Sendai are commercially available [e.g., Wellferon from GlaxoSmithKlein]. In addition, natural IFNs are also produced from fresh isolated PBMCs infected with Sendai virus (e.g., MultiFeron from Viragen Inc). Because EBV is positive in more than 90% of populations, it is almost certain that the fresh isolated PBMCs contain EBV-positive cells. Therefore, the cells infected with EBV and the induction of IFN by Sendai virus can be used together for commercial IFN production. Finally, some “personalized” cell therapies have been reported in which patient’s cells were isolated, treated, expanded, and finally injected back into the patient [23]. It is in essence identical to personalized IFN treatment. Thus, personalized IFNs may be approvable by regulatory agencies for medical usage.

This system might also expandable to other personalized treatments. From personal genome projects, it is apparent that all humans have certain unique gene sequences. Also, post-translational modifications might also be different among individuals. If a person has a unique sequence in the target gene, or post-translational modifications of the gene products are important for their functions, it might be wise to use the personalized system to generate unique gene products for the individual to optimize the patient’s preventative and/or therapeutic care.

In summery, we have established a system for the production of personalized IFNs. However, the superiority of personalized IFNs has to be demonstrated *in vivo* in patients in future studies. It is of note that some small modification may generate some big effect *in vivo*. IFN therapy of melanoma patients increases the survival rate by about 10% [24]. A slightly improvement here might have a big impact for therapeutic outcomes.

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GLOSSARY

IFN	Type I interferons
EBV	Epstein-Barr virus
LMP-1	EBV latent membrane protein 1
IRF-7	IFN regulatory factor 7
HCV	hepatitis C virus

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Figure 1. Personalized type I IFN production system

Step 1: Blood collection. A small amount (5-10 ml) of blood will be drawn from an individual. The PBMC will be isolated from the blood. Step 2: EBV transformation. The PBMC will be infected by EBV, and the resulting immortalized B cells, lymphoblastoid cell lines (LCL), will grow to large volume in a tissue culture system. Step 3: IFN induction and purification. EBV-transformed cells are primed to produce robust levels of IFNs upon virus infection. IFNs can be purified. Step 4: IFN administration. IFNs can be administered back to the individual. Thus, the IFNs from a specific individual's cells can be manufactured and consumed by the same person.

Figure 2. Generation of personalized IFNs in laboratory scale

A) The production of IFN- α by personalized cell lines. PBMCs were isolated from fresh buffy coats and EBV was inoculated with the PBMC (10^7 cells/ml) in the presence of cyclosporine in 96-well plates. The cells were fed by adding 50% of fresh medium at weekly intervals, and transformation was judged by outgrowth of cells over 3-5 weeks of culture. Personalized LCLs from three individuals (named Person A, B, or C) were infected by Sendai virus (Spafas, Inc; 200 HA units per ml) for seven hours. The IFN- α in supernatants was measured with the use of ELISA (PBL Biomedical Laboratories; catalog number 41100). Standard deviations are shown. +/-: with or without Sendai infection. B) Production of IFN- β from personalized cell lines. The personalized cell lines were infected by Sendai virus. Total RNAs were isolated and used for RPA with IFN- β and GAPDH probes. Specific protections of IFN- β and GAPDH RNAs are indicated. C) Comparable amounts of IFN- α can be produced from IB4 and Namalwa cell lines. IFNs are induced by infection of Sendai virus (200 HA units per ml) for seven hours. The cell culture media were collected, and the

concentrations of IFN- α were measured with the use of ELISA. IFN- α production in IB4 was set as 100. IFN productions in PBMCs are varied among individuals.