High efficiency gene transfer is an efficient way of defining therapeutic targets: a functional genomics approach

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Abstract

Background—Dendritic cells are the most potent antigen presenting cells and key to many aspects of the immune function. Studying the intracellular signalling mechanism used by dendritic cells would provide an insight into the functioning of these cells and give clues to strategies for immunomodulation.

Method—Highly efficient adenoviral infection of dendritic cells for the delivery of transgenes was obtained. These viral vectors were used to introduce IkBα into dendritic cells for the inhibition of NF-κB. This was used to investigate the role of NF-κB in dendritic cell function.

Results—By blocking the NF-κB function a potent inhibition of the expression of costimulating molecules by dendritic cells with the concomitant loss of T cell stimulating function was demonstrated.

Conclusion—The use of adenoviral vectors may be a useful way of studying the role of genes in dendritic cell function.

Now that the human genome is sequenced,1 2 we know the sequence of almost all 30 000 or so genes, and many of the single nucleotide polymorphisms in the vicinity of these genes. It is these genes that encode the function of 100 000 or so proteins, and the differences in gene functions that define the alterations between health and disease. A key question is to match genes and proteins to the pathogenesis of disease and define new therapeutic targets, which may be targets for treatment to convert the functions typical of disease back towards a semblance of normality. Here we review our work, which defines new targets of possible relevance to the autoimmunity of joints, defining genes controlling the function of dendritic cells (DC).3 Yoshimura, unpublished data).

DC are the most efficient antigen presenting cells (APC) for activating naive and primed T cells.4 Hence, DC have a key role in inducing the activity of appropriate T cells in response to varying environmental challenges. DC inactivation would help to reduce immune responses, such as those occurring in autoimmune diseases like rheumatoid arthritis (RA) and, clearly, DC activation would be important for increasing the efficacy of vaccines. Vaccines have been the most cost effective therapeutic intervention since they were discovered by Jenner over 200 years ago.5

Materials and methods

REAGENTS

Human recombinant granulocyte macrophage colony stimulating factor (GM-CSF) and tumour necrosis factor a (TNFα) were kind gifts of Dr Glenn Larsen (GI) and Dr D Tracey (BASF), respectively. Human recombinant interleukin 4 (IL4) was purchased from R&D Systems (Minneapolis, MN).

DENDRITIC CELL MATURATION

Mononuclear cells were isolated from single donor plateletphoresis residues as described.6 A total of 10⁶ monocytes were plated in 10 ml volumes in 100 mm Petri dishes, and dendritic cell maturation was performed as described.7 On day 6, non-adherent cells were collected and analysed, or transferred to new Petri dishes. The cultures were supplemented with monocyte conditioned medium8 and TNFα at final concentrations of 20% v/v and 10 ng/ml, respectively. Fresh GM-CSF and IL4 were present throughout the culture period. In some experiments, cells were washed out of supplemental cytokines or monocyte conditioned medium at day 6 or day 9 before use in phenotypic or functional assays.

ADENOVIRAL VECTORS

Recombinant, replication deficient, adenoviral vectors encoding E coli β-galactosidase (AdvβGal) or having no insert (Adv0) were provided by Drs A Byrnes and M Wood (Oxford, UK). An adenovirus encoding porcine IkBα with a cytomegalovirus promoter and a nuclear localisation sequence (AdvIkBα) was provided by Dr R de Martin (Vienna, Austria).9 Viruses were propagated and titred as previously described.10 11

FLOW CYTOMETRY ANALYSIS OF DC

INFECTIBILITY AND SURFACE MARKERS

All samples were analysed on a FACSscan flow cytometer using the CellQuest software (Becton Dickinson, San José, CA). Analysis was carried out on a population of cells gated by forward and side scatter to exclude dead cells and debris. Dendritic cell surface markers were studied using monoclonal antibodies to HLA-DR, CD86, and CD25 (phycoerythrin (PE) conjugated; Pharmingen), HLA-DQ (FITC conjugated; Pharmingen), CD 80 (PE conjugated; Pharmingen).
Results

ADENOVIRUS CAN INFECT ~100% OF MATURE DENDRITIC CELLS

Although it is immature DC that are specialised for antigen uptake, it is mature DC that are the most active antigen presenters. Thus one technical problem was to resolve whether mature DC would take up adenovirus efficiently enough (>80%) for inhibition of their function to be readily measured so that pathways regulating DC could be studied. Reports of infectability have been published, but not close to this level.12 13

However, we found that mature human DC, generated by the method of Sallusto and Lanza Vecchia,14 using GM-CSF and IL4 were infectible by adenovirus. When a multiplicity of infection of 300:1 adenoviruses for a cell was used, all DC seemed to be infected, as judged by a variety of assays, initially flow cytometry and microscopy. This was subsequently confirmed by functional and biochemical assays. In the situation shown in fig 1, a virus encoding IκBα (AdvIκBα) was used to infect DC. Infection with this virus resulted in a large increase in the expression of IκBα and inhibition of lipopolysaccharide-induced NF-κB.

This work, summarised in fig 1, was recently reported by Yoshimura et al.15

ADENOVIRUSES ENCODING IκBα INTERFERE WITH ANTIGEN PRESENTATION IN THE MIXED LYMPHOCYTE RESPONSE

The mixed lymphocyte response is accepted as an in vitro model of a strong primary response, as seen in allotransplantation. It is much more convenient to study, using human cells, than other T cell responses. Thus it was chosen to evaluate DC antigen presentation in view of the fact that DC are the most powerful cells to induce a mixed lymphocyte response.15 As few as 30 DC can induce a measurable response from 10^4 T cells, as shown in fig 2.

The results of a comparison of AdvIκBα and Adv0, with normal uninfected DC are shown in fig 2. This demonstrates the dramatic effect of down regulating DC function by AdvIκBα. This may be surprising if one believes that adenoviruses are highly proinflammatory and, if so, it might be suspected that at least Adv0 should have activated DC.1 The dramatic diminution of DC function by AdvIκBα suggests that the staining results, which indicate that >90% of cells are infected, are probably correct. Furthermore, they show that the NF-κB family15 of transcription factors (five subunits) controls the antigen presenting function of DC. Which of these subunits is critical to DC function cannot be determined if so, it might be suspected that at least Adv0 should have activated DC.1 The dramatic diminution of DC function by AdvIκBα suggests that the staining results, which indicate that >90% of cells are infected, are probably correct. Furthermore, they show that the NF-κB family15 of transcription factors (five subunits) controls the antigen presenting function of DC. Which of these subunits is critical to DC function cannot be determined as a large excess of IκBα would inhibit them all. As 100 normal or Adv0 infected DC were highly active in the mixed lymphocyte response, the fact that 10^4 AdvIκBα infected DC were not stimulatory was interesting, demonstrating a profound immune inhibition.

MECHANISMS OF ADVIκBα INHIBITION OF DC FUNCTION

The marked efficiency of inhibition by AdvIκBα made it imperative to understand the mechanisms by which it was mediated. As several sets of molecular mechanisms play a part in the antigen presenting function, these were all examined.

Peptides bound to the major histocompatibility complex (MHC) are the target of recognition by the T cell receptor for antigen. Hence, the degree of MHC expression has long been a major factor in APC function.17 By flow cytometry, it was shown that the HLA class II
Gene transfer

Figure 2  Mature dendritic cells (DC) were left uninfected, infected with Adv0, or infected with AdvIkBa at a multiplicity of infection of 300. DC were then plated in graded doses for 10 hrs and measured for BrdU uptake (CD40+49 nm) A and [3H]thymidine ([3H]Tdr) uptake (B). Each point represents the mean (SEM) of either six (A) or three (B) separate experiments.

Discussion

It is widely believed that DC are important in the induction of human autoimmune diseases. This was postulated for RA by many authors—Zvaifler et al, Thomas and Lipsky, and for other diseases, by Drakesmith et al. Our own studies in human Graves' disease had first proposed that up regulated antigen presenting function was important in its pathogenesis. In that example, we considered that it was the epithelial cells that were critical, as they were HLA-DR expressing and made all the specific antigens.

Our studies, recently reported by Yoshimura et al (Yoshimura, 2001 in press) and summarised here, indicate that NF-κB is an essential transcription factor for regulating DC APC function. Previous workers (for example Pettit et al) had shown that NF-κB was activated in mature DC, but this, in itself, does not demonstrate their pivotal role. A variety of drugs have been used, and the suggestion has been made that NF-κB is important, but the non-selectivity of these drugs, such as cyclosporin, precludes such a conclusion. Our work with a specific adenovirus inhibitor of NF-κB confirms and proves the previous suggestions.

There is another important potential mechanism to explain the virtually complete inhibition of the T cell response—namely, immunological tolerance. We have demonstrated this in a companion study using the proteosome inhibitor PSI, which also blocks DC APC function (Yoshimura, 2001 in press). This result is compatible with several recent studies, which indicate that immature DC are effective tolerogens.

The studies reported here, together with previous work, highlight the importance of NF-κB in the molecular pathogenesis of autoimmune diseases. This includes RA, where we have previously shown that AdvIkBa down regulates many of the pathological functions of the rheumatoid synovium—it inhibits the synthesis of the proinflammatory cytokines, especially TNFα and IL6, without interfering with the inhibitory cytokines, such as IL10. It also down regulates the production of matrix metalloproteinases, without down regulating the inhibitors—for example, TIMP-1.

NF-κB inhibition would block dendritic and macrophage function in RA synovium and, hence, would have an interesting therapeutic potential.
profile. However, such drugs might interfere with too many biological processes to be safe. The use of adenoviruses permits further refinements to therapeutic targeting—for example, it may be possible to inhibit only a subset of NF-κB, using, for example, antisense to relB or a truncated relB. This might disclose a therapeutic target which blocks only some NF-κB functions and might, thereby, still be effective and safer than blocking all NF-κB.

It is impossible to predict the outcome of NF-κB blockade in humans with different diseases. Would the benefit outweigh the risk? However, it is possible to predict that adenoviruses are a highly useful tool for efficient gene transfer into normal human cells and cells from pathological sites and, therefore, allow the evaluation of the effects of blocking pathways in humans. That should accelerate the research needed to convert our recent therapeutic advances in RA, into an eventual cure.

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**Figure 3** The effect of AdvκBα infection on dendritic cell antigen presenting and costimulatory molecules as well as the costimulatory cytokine interleukin 12 (IL12) was studied. κBα was overexpressed in mature DC for two days and then cells were collected and stained by FACS for HLA (A) or CD80/86 (B), or stimulated by soluble CD40L for 24 hours, and then IL12 production was assayed by enzyme linked immunosorbent assay (ELISA) (C).

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9 Wrighton CJ, Hoeser-Wurbinke R, Moli T, Eyfner R, Bach FH, de Martin R. Inhibition of endothelial cell activation...


