IL-4 deficiency does not impair the ability of dendritic cells to initiate CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses in vivo

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Abstract

Several reports have described a role of IL-4 in dendritic cell function. We have examined the number and phenotype of dendritic cells from C57Bl/6 wild-type and IL-4<sup>−/−</sup> mice, and compared their ability to induce T cell immune responses in vivo and in vitro. We observed that the number of dendritic cells in the spleens and lymph nodes of IL-4<sup>−/−</sup> mice is comparable to the number found in wild-type mice. In addition, the expression of maturation markers such as MHC II, CD40, CD80 and CD86, and of differentiation markers such as CD4, CD8 and CD11b, was also comparable in the two populations. Splenic wild-type and IL-4<sup>−/−</sup> dendritic cells were both able to present antigen to T cell receptor transgenic CD4<sup>+</sup> or CD8<sup>+</sup> T cells in culture. When pulsed with antigen in vitro and then injected subcutaneously into C57BL/6 host mice, both populations of dendritic cells were able to induce the division of T cell receptor transgenic CD4<sup>+</sup> or CD8<sup>+</sup> T cells in vivo. This was the case regardless of whether the antigen used in these experiments was a low or a high affinity T cell receptor ligand. Similarly, both populations of dendritic cells were able to activate antigen-specific cytotoxic T cell responses and initiate tumor-protective immune responses in vivo. We conclude that IL-4<sup>−/−</sup> and wild-type dendritic cells have a comparable ability to initiate T cell immune responses when in an IL-4-sufficient environment.

Introduction

The cytokine IL-4 has multiple effects on immune responses, of which the most notable are the ability to direct CD4<sup>+</sup> T cells development towards 'type 2' cells which secrete IL-4, IL-5 and IL-13, and the ability to induce the switching of B cells to IgE secretion (1,2). Thus, IL-4 has been principally regarded as a key factor in the initiation of type 2 immune responses. In addition to the effects described, other effects of IL-4 have also been observed which extend beyond its recognized role in T helper 2 (Th2) immune responses. Somewhat paradoxically, IL-4 appears necessary for the development of cytotoxic T lymphocyte (CTL) responses and type 1 Th immune responses. For example, the presence of IL-4 promotes the development of Th1 and CTL responses to tumors (3,4) and can increase resistance to Leishmania infection if administered early during infection (5). Furthermore, IL-4 is also critical for the activation of allorreactive T cells in vitro, and for the rapid rejection of histoincompatible skin grafts in vivo (6). These effects of IL-4 on type 1 responses appear to be mediated through antigen-presenting cells and an increased secretion of IL-12. IL-4 has been demonstrated to directly increase IL-12 secretion by dendritic cells (DC) in vitro (7) and in vivo (5). Increased IL-12 in turn allows the differentiation of CD4<sup>+</sup> T cells into IFN-γ-secreting Th1 cells (8), the activation of CD8<sup>+</sup> T cells into potent effector cells (9) and prevents CD8<sup>+</sup> T cell anergy (10). In all these systems, IL-4 appears to be mainly derived from CD4<sup>+</sup> and CD8<sup>+</sup> T cells (4,11), but secretion of IL-4 from other cell types has also been observed (12). Altogether, these observations suggest a critical function of IL-4, not only in Th2 immune responses, but in most T cell mediated immune responses as well.

In contrast to some of the observations above, other authors have reported that IL-4 can have an inhibitory effect on CD8<sup>+</sup> T cells and immune responses (13). Infection with a recombinant vaccinia virus induced stronger CD8<sup>+</sup> T cell activation in IL-4<sup>−/−</sup> mice compared to WT mice (14). In addition, IL-4 has been shown to modulate expression of costimulatory molecules on DC (15), which correlated with a strongly decreased incidence of diabetes in transgenic mice where IL-4 secretion had been targeted to the pancreas using the rat insulin promoter.
IL-4-deficient dendritic cells

The antigen-presenting function of DC therefore appears to be regulated by IL-4 through its effects on expression of costimulatory molecules and production of IL-12. It is possible that defects in the activity of DC may also contribute to the multiple defects in immune responses described above, which affected both CD4+ and CD8+ T cell responses. However, no reports to date have directly addressed whether the presence of IL-4 during DC development affects the phenotype or function of DC. To address this point, we compared the ability of DC from WT and IL-4−/− mice to activate T cell responses in vitro and in vivo, and find that this appears completely normal.

Methods

Mice

C57BL/6 mice were obtained from Jackson Laboratories and were maintained at the Biomedical Research Unit of the Wellington School of Medicine. IL-4−/− mice were originally obtained from Dr Manfred Kopf (16). The ‘line 318’ mouse strain, transgenic for a TCR specific for H-2Dk + fragment 33–41 of the lymphocytic choriomeningitis virus (LCMV) glycoprotein (LCMV33–41) was kindly provided by Dr H. Pircher (Institute of Medical Microbiology, University of Freiburg, Germany). OT-II mice, transgenic for a TCR specific for I-Ak + OVA, were kindly provided by Dr Bill Heath (WEHI, Melbourne, Australia). All experimental protocols were approved by the Wellington School of Medicine Animal Ethics Committee and performed according to Institutional guidelines.

In vitro culture media and reagents

Unless otherwise stated, all cultures were maintained in complete medium comprising Iscove’s modified Dulbecco’s medium and 30 mM EDTA; cells were recovered by centrifugation and resuspended at 2 × 10^7 cells/ml in complete medium. Cells were pulsed with 1 μCi/well of [3H]thymidine for the final 8 h of a 48 h culture, and then harvested for liquid scintillation counting.

DC preparation

Spleens were harvested and incubated for 30 min at 37°C in collagenase type II (Invitrogen, Auckland, NZ) and DNase (Sigma, St Louis, MO, or Roche Diagnostics NZ Ltd, Auckland, NZ). Digestion was stopped by adding FBS supplemented with 30 mM EDTA; cells were recovered by centrifugation and enriched for low density mononuclear cells using a 1,077 g/cm3 gradient (Nycospre, Nycomed, Oslo, Norway, or 65% Percoll, Amersham Biosciences, NZ). In some experiments, B cells were depleted using sheep anti-mouse IgG Dynabeads (Dynal, Victoria, Australia). Fc receptors were blocked using 2.4G2, and CD11c+ cells were labeled with N418–biotin (affinity purified from culture supernatants) or HL-3–biotin (Pharmingen, San Diego, CA) and Dynabeads conjugated to streptavidin, and Dynabeads conjugated to rat anti-mouse IgG (Dynal, Victoria, Australia). Purified CD8+ T cells were prepared by depleting lymph node cell suspensions of CD4+ T cells and B cells using GK1.5–biotin and magnetic depletion with Dynabeads.

T cell proliferation in vitro

Purified CD4+ T cells were prepared from lymph node cell suspensions by depleting CD8+ and B cells using 2.43–biotin and Dynabeads conjugated to streptavidin, and Dynabeads conjugated to rat anti-mouse IgG (Dynal, Victoria, Australia). Purified CD8+ T cells were prepared by depleting lymph node cell suspensions of CD4+ T cells and B cells using GK1.5–biotin and magnetic depletion with Dynabeads. Thymidine incorporation was measured by culturing 1–2 × 10^5 CD4+ T cells from OT-II mice, or CD8+ T cells from line 318 mice, with various numbers of antigen-loaded DC in 96-well culture plates in complete medium. Cells were pulsed with 1 μCi/well of [3H]thymidine for the final 8 h of a 48 h culture, and then harvested for liquid scintillation counting.

Carboxy-fluorescein diacetate succinimidyl ester (CFSE) labeling

Single cell suspensions were prepared from spleen and lymph nodes by teasing through nylon gauze, and erythrocytes were lysed in 0.14 M NH4Cl and 17 mM Tris–HCl. Cells were washed and resuspended at 2 × 10^7 cells/ml in phosphate-buffered saline (PBS) at room temperature. An equal volume of 2.5 μM CFSE (Molecular Probes, Eugene, OR) in PBS was added to the cell suspension, immediately vortexed, and incubated for 8 min at room temperature. Unbound dye was quenched by the addition of an equal volume of PBS and then washed three times with ice-cold medium containing 5% FBS.

T cell division in vivo

CFSE-labeled lymph node and spleen cell suspensions from OT-II mice or line 318 mice, containing a total of 0.7 × 10^6 Vα2+Vβ5.1+ transgenic T cells (OT-II) or 1 × 10^6 Vα2+Vβ8+ transgenic T cells (line 318), were injected intravenously (i.v.) into C57Bl/6 mice in a total volume of 0.3 ml. One day later, the mice were immunized with 1 × 10^5 antigen-loaded DC subcutaneously (s.c.) into the anterior forelimb. Control mice received DC only. Cells from the draining axillary and brachial lymph nodes were harvested 72 h after immunization.

FACS analysis

Anti-TCR (2.4G2), anti-MHC Class II (3JP), anti-CD4 (GK1.5), anti-CD11c (N418) and anti-CD86 (GL-1) antibodies were affinity purified from culture supernatants of the relevant B cell hybridomas using protein G-Sepharose (Pharmacia Biotech, Uppsala, Sweden) and conjugated to biotin, FITC or
been modified to express a minigene encoding LCMV 33–41 of the Lewis lung carcinoma LLTC (C57BL/6, H-2b) which has been introduced via electroporation into the skin of WT and IL-4−/− mice. Tumor protection assays were performed in the non-immunized control group, and experimental groups were adjusted accordingly.

In vivo cytotoxicity assays
Cytotoxicity was assessed on fluorescence-labeled syngeneic spleen cell populations administered by i.v. injection into groups of immunized mice (n = 4–5) and a non-immunized control group (n = 3). The target cell preparation contained equal proportions of two differentially labeled populations: a control population labeled with 10 μM chloromethylbenzoylaminotetramethylrhodamine (CMTMR, Molecular Probes, Eugene, OR), and a population labeled with 1.25 μM CFSE and loaded with 10 μM LCMV-GP33–41 or OVA257–264 peptide. Cytotoxicity was assessed 20 h after target cell administration by FACS analysis of lymph node tissue preparations from inguinal and axillary nodes, and is presented as percent killing calculated using the formula \[ \text{percent killing} = \frac{1 - \text{targets with peptide/targets without peptide}}{100}. \] Any variability in proportion of cells in the different target populations was assessed in the non-immunized control group, and experimental groups were adjusted accordingly.

Tumor protection assays
Groups of C57BL/6 mice (n = 5) were immunized by s.c. injection into the flank with 10⁵ or 10⁴ DC loaded with 10 μM LCMV33–41 peptide. Control animals received DC only. All animals were challenged with 10⁶ LL-LCMV tumor cells administered by i.v. injection into the flank with 10⁵ or 10⁴ DC loaded with 10 μM LCMV-GP33–41 or OVA257–264 peptide. Cytotoxicity was assessed 20 h after target cell administration by FACS analysis of lymph node tissue preparations from inguinal and axillary nodes, and is presented as percent killing calculated using the formula \[ \text{percent killing} = \frac{1 - \text{targets with peptide/targets without peptide}}{100}. \] Any variability in proportion of cells in the different target populations was assessed in the non-immunized control group, and experimental groups were adjusted accordingly.

Results

Numbers and phenotype of DC in IL-4−/− mice
The percentages of DC in the secondary lymphoid organs of WT and IL-4−/− mice were examined. Spleens and lymph nodes were harvested from WT and IL-4−/− mice, and total peripheral blood mononuclear cells (PBMC) were stained with antibodies specific for a number of DC surface markers. Figure 1 shows representative stainings of these cell populations. As shown in the top row of Fig. 1, spleen cell suspensions from WT and IL-4−/− mice contained comparable proportions of CD11c⁺ cells, with 1.39 ± 0.12% CD11c⁺ cells in WT mice, and 1.25 ± 0.29% in IL-4−/− mice.

DC comprise a heterogeneous population of cells expressing different surface markers (18). The distribution of DC into different subpopulations was compared in WT and IL-4−/− mice. As shown in Fig. 1, the CD11c⁺ population from the spleens and lymph nodes of WT and IL-4−/− mice comprised comparable proportions of cells expressing the DC subpopulation markers CD11b, CD8 and CD4. The expression of other markers associated with DC function, MHC II, CD40, CD80 and CD54, were also compared using density-gradient enriched spleen DC populations. As shown in Fig. 2, each of the above markers was expressed at a comparable level on splenic DC from WT or IL-4−/− mice.

IL-4−/− DC can stimulate T cell responses in vitro and in vivo
To establish whether the ability of DC to initiate immune responses was compromised by development in an IL-4−/− deficient environment, we assessed the ability of WT and IL-4−/− splenic DC to initiate immune responses in vitro and in vivo. Splenic DC were isolated by positive selection using anti-CD11c antibodies and magnetic beads, incubated with the relevant antigens for 2 h, and then cultured for 2 days with specific CD4⁺ or CD8⁺ cells purified from the lymph nodes of TCR transgenic mice. The WT and IL-4−/− DC populations used in these studies were of comparable purity in each experiment, and comprised similar proportions of CD8⁺ and CD4⁺ cells (data not shown). Antigen-specific CD4⁺ T cells were obtained from OT-II mice, which carry a TCR specific for OVA323–339, or OVA257–264 peptide. To induce the proliferation of CD4⁺ OT-II cells, DC were incubated in the presence of the minimal OVA epitope OVA257–264, or in the presence of whole OVA protein (OVAp), for 2 h before the assay. As shown in Fig. 3, at every cell concentration tested, DC from WT or IL-4−/− mice induced similar proliferation of OT-II T cells. This was the case regardless of whether OVAp or the minimal OVA epitope OVA257–264 were used as antigens. Figure 3 shows a similar experiment carried out to compare the proliferation of CD8⁺ T cells to antigen presented on WT or IL-4−/− DC. In this case, DC were incubated in the presence of the synthetic peptide LCMV-GP33–41, which is a high affinity agonist for the line 318 TCR, or the substituted peptide A4Y, which is a weak agonist. Although T cell proliferation to the weak agonist A4Y was much lower than to the agonist LCMV-GP33–41, undistinguishable thymidine incorporation was observed when using WT or IL-4−/− DC. Together these results indicate that the two populations of DC express comparable levels of MHC and costimulatory molecules, and have comparable abilities to process antigen (in the case of OVAp).

Induction of in vivo immune responses may have more stringent requirements than induction of in vitro proliferation, as both APC and T cells are required to migrate to the appropriate site where interaction can occur. To evaluate the
T cell response in vivo we monitored cell division of antigen-specific T cells labeled with CFSE, where CFSE fluorescence can be observed to halve at each consecutive cell division. TCR transgenic T cells were injected i.v. on day 0, and antigen-loaded DC were administered s.c. on the following day. T cell division was evaluated in the draining lymph nodes at 72 h after DC administration, as this time was found to be optimal in pilot experiments. As shown in Fig. 4, CD4+ OT-II cells underwent a comparable number of divisions regardless of whether antigen was presented in the context of a WT or an IL-4-/- DC. Similarly, CD8+ line 318 T cells divided a similar number of times after immunization with antigen presented on WT or IL-4-/- DC.

Again, we considered the possibility that the use of relatively high affinity TCR ligands, such as LCMV-GP33-41 for line 318 T cells, may fail to reveal smaller differences in the ability of IL-4-/- DC to induce T cell responses. We therefore used peptide A4Y which is a weak agonist for line 318 T cells. As shown in Fig. 4, using A4Y peptide resulted in a much reduced accumulation of divided T cells in vivo. However, no difference between WT and IL-4-/- DC became apparent in those experiments.

**Induction of effector CD8+ T cell responses by WT and IL-4-/- DC**

T cell division as evaluated by CFSE staining is a sensitive measure of early T cell responses to antigen recognition, but does not necessarily reflect the generation of effector cells (19). In order to establish whether early T cell division had been followed by generation of effector T cells, we evaluated the development of cytotoxic activity in C57Bl/6 mice that had been immunized s.c. with WT or IL-4-/- DC that had been coated with LCMV-GP33-41 or OVA257-264 peptide. Unlike the experiments in the previous paragraph, these experiments
were carried out in C57Bl/6 mice that had received no adoptive transfer of TCR transgenic T cells, thus reflecting the generation of CTL from a naive repertoire. To measure cytotoxic activity, 1 week after immunization, mice were injected i.v. with target spleen cells that had been labeled with CFSE and loaded with LCMV-GP 33–41 or OVA 257–264 peptide, or labeled with CMTMR and loaded with no peptide. The differential survival of these target spleen cells in vivo was evaluated 48 h after target cell transfer. Target cell recoveries were evaluated in immunized mice and compared to recoveries in naive C57Bl/6 mice to minimize confounding effects of the dye label on target survival. As shown in Fig. 5, cytotoxic activity was readily demonstrated in immunized mice. No difference was apparent in the LCMV-GP 33–41-specific CTL activity induced by WT or IL-4−/− DC immunization, even when limiting numbers of DC were used. In addition, IL-4−/− DC were also able to induce OVA257–264-specific CTL, although the CTL activity was somewhat lower than in mice immunized with WT DC. Therefore, WT and IL-4−/− DC were both able to induce CTL differentiation in vivo.

As a further measure of the ability of DC to activate effector T cell responses, we also evaluated their capacity to induce tumor-protective immunity. We therefore immunized C57Bl/6 mice with LCMV-GP 33–41 peptide-loaded DC prepared from WT or IL-4−/− mice, and evaluated their ability to resist a challenge with LCMV-GP 33–41-expressing tumor cells.
administered 1 week later. As shown in Fig. 6(A), these freshly prepared DC induced only weak or undetectable anti-tumor immune responses. In the attempt to induce more powerful anti-tumor immune responses, we used DC that had been activated by overnight adherence to plastic. This method of purification also yielded similar numbers of WT and IL-4−/− DC, which had similar surface phenotypes. As shown in Fig. 6(B), both DC populations could induce tumor protective immunity in recipient mice. When lower numbers of DC were used to immunize mice, no effect on tumor growth could be detected (data not shown). We conclude that both WT and IL-4−/− DC are able to induce the activation of effector T cells with comparable tumor-protective activity.

Conclusions

Several reports have described a role for IL-4 in CD4+ T cell responses, CTL generation and DC function. IL-4 appears necessary for the early production of IL-12 by DC (7). Lack of IL-4 production causes altered resistance to infection with the protozoan parasite Leishmania major (5) and defective CTL generation after vaccination with a recombinant protein (12). T cells have been identified as the source of IL-4 in some of these experiments (4), but other cell types, including monocytic cells, have also been implicated (12). Although DC are critical components of the immune response, and effects of IL-4 on their function have been observed (7,15), the properties of DC in IL-4−/− mice have not been directly addressed.

In this report we compare the ability of WT and IL-4−/− DC to induce the early activation of CD4+ and CD8+ T cells in vitro and in vivo, and initiate tumor-specific CD8+ effector T cells in vivo. In most experiments we used DC that were freshly isolated from murine spleen, and had not been activated by deliberate exposure to infectious stimuli or adherence to plastic. DC were loaded with synthetic peptide antigen and injected into host mice where their ability to induce specific immune responses

Driven by the need to boost the immune system against cancer, researchers have explored the potential of dendritic cells (DCs), a type of immune cell that plays a critical role in initiating and regulating immune responses. In a recent study, investigators aimed to enhance the anti-tumor immune responses by using DCs that had been activated by overnight adherence to plastic. This method of purification yielded similar numbers of WT and IL-4−/− DC, which had similar surface phenotypes.

The study concluded that both WT and IL-4−/− DC were capable of inducing the activation of effector T cells with comparable tumor-protective activity. This finding suggests that IL-4−/− DC can be used as an alternative strategy to boost anti-tumor immune responses.

The conclusion further highlights the importance of DCs in the immune response and emphasizes the potential of IL-4−/− DC as a therapeutic approach in cancer immunotherapy.
mediated in large part by secretion of IFN-γ-activated by overnight culture. We have previously shown that tumor responses required the use of DC that had been induced by the two types of DC were also similar, although anti-proliferation. In addition, CTL activity and anti-tumor activity was as in (A), with the exception that DC were prepared by collagenase digestion and differential plastic adherence during overnight culture before antigen loading and injection. The WT and IL-4-deficient DC populations used comprised 39% CD11c<sup>high</sup> cells for the WT DC population and 41% for the IL-4<sup>−/−</sup> DC population. Seven days after antigen injection, mice were challenged with tumor cells, and tumor growth evaluated by measuring the two perpendicular tumor diameters with a calliper. Results are expressed as the mean product of tumor diameters ± SE. (B) Experimental set-up was as in (A), with the exception that DC were prepared by collagenase digestion and differential plastic adherence during overnight culture before antigen loading and injection. The WT and IL-4<sup>−/−</sup> DC populations used for immunization contained 85.6% and 85.2% CD11c<sup>high</sup> cells, respectively. Results are expressed as the mean product of tumor diameters ± SEM.

was evaluated. Our experiments revealed no defect in the ability of these DC to initiate either CD4<sup>+</sup> or CD8<sup>+</sup> T cell proliferation. In addition, CTL activity and anti-tumor activity induced by the two types of DC were also similar, although anti-tumor responses required the use of DC that had been activated by overnight culture. We have previously shown that in our model, protective immunity to tumor challenge is mediated in large part by secretion of IFN-γ by tumor-specific T cells, as IFN-γ<sup>−/−</sup> mice are unable to resist tumor challenge (20) but they are able to generate effective CTL responses after DC immunization (our unpublished observation). In addition, we have also shown that generation of effective anti-tumor activity requires CD4<sup>+</sup> T cell help (21). Together, our data indicate that the presence of IL-4 in the environment was sufficient to provide the required set of stimuli for the activation of CD4<sup>+</sup> T cells and the provision of T cell help, and for full CD8<sup>+</sup> T cell activation to IFN-γ secretion and effector function.

The injection of WT and IL-4<sup>−/−</sup> DC into WT mice could fail to reveal differences between the two types of DC, if antigen was transferred from the injected DC to host DC. However, we do not think this is a possible explanation for our results. Our <em>in vivo</em> experiments used DC coated with minimal MHC I-binding peptides, while cross-presentation requires the presence of protein in donor cells (22, 23). Indeed, peptide-loaded WT DC induced comparable division of line 318 TCR transgenic T cells that had been transferred into C57Bl/6 or bm13 recipients (D. Ritchie and F. Ronchese, unpublished data), indicating that the response was mostly or entirely due to antigen presented on donor DC. We therefore conclude that the CD8<sup>+</sup> T cell responses assessed in this study reflect the antigen-presenting capacity of the injected WT or IL-4<sup>−/−</sup> DC.

In contrast to the situation for CD8<sup>+</sup> T cells, antigen transferred to host DC may contribute to the induction of CD4<sup>+</sup> T cell responses by injected DC (24). Again, the observations that anti-tumor immune responses require CD4<sup>+</sup> T cell help (21), and that CD4<sup>+</sup> and CD8<sup>+</sup> T cells must recognize antigen on the same APC (25) suggest that endogenous APC would have little role in most of the responses described in this paper.

Recent reports have indicated that IL-12 is required for the acquisition of effector function by CD8<sup>+</sup> T cells <em>in vitro</em>, and suggested that IL-12 acts as a ‘signal 3’ for the activation of CD8<sup>+</sup> T cells (9, 10). Secretion of IL-12 by DC requires exposure to IL-4 (7). Because effector CD8<sup>+</sup> T cell responses were elicited regardless of whether WT or IL-4<sup>−/−</sup> DC were used in our experiments, we conclude that sufficient amounts of IL-12 were likely to be present to allow full CD8<sup>+</sup> T cell activation. We therefore conclude that the presence of IL-4 during the final stages of DC maturation is sufficient for the full antigen presenting function of these cells.

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**Abbreviations**

CFSE carboxy-fluorescein diacetate succinimidyl ester  
CMTMR chloromethyl-benzoylamino-tetramethylrhodamine  
DC dendritic cell  
i.v. intravenous  
LCMV lymphocytic choriomeningitis virus  
OVA chicken ovalbumin  
OVA<sub>p</sub> OVA protein  
s.c. sub-cutaneous  
WT wild type

**References**

8. Hochrein, H., O'Keeffe, M., Luft, T., Vandenaabie, S., Grumont, R. J., Maraskovsky, E. and Shortman, K. 2000. Interleukin (IL)-4 is
a major regulatory cytokine governing bioactive IL-12 production by mouse and human dendritic cells. J. Exp. Med. 192:823.
11 Carvalho, L. H., Sano, G., Hafalla, J. C., Morrot, A., Curotto de Lafaille, M. A. and Zavala, F. 2002. IL-4-secreting CD4+ T cells are crucial to the development of CD8+ T-cell responses against malaria liver stages. Nat. Med. 8:166.